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BIOFILM COMPOSITION AND FUNCTION IN STORMWATER CONSTRUCTED WETLAND SYSTEMS ON THE SWAN COASTAL PLAIN, WESTERN AUSTRALIA.

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BIOFILM COMPOSITION AND FUNCTION IN STORMWATER CONSTRUCTED WETLAND SYSTEMS ON THE SWAN COASTAL PLAIN, WESTERN AUSTRALIA.

BY

S.A. Hawkins

A Thesis Submitted in Partial Fulfillment
of the Requirements for the Award of
Bachelor of Science (Environmental Management) Honours
at the Faculty of Communications, Health and Science,
Edith Cowan University, Western Australia.

DATE OF SUBMISSION: 28 APRIL, 2000.

Supervisors: Dr Paul Lavery and Dr Mark Lund,
School of Natural Sciences
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WESTERN AUSTRALIA

ABSTRACT

The ability of natural wetlands to act as effective nutrient sinks and to absorb new nutrient loadings is well documented. Constructed wetland systems (CWSs) aimed at optimising these nutrient removal mechanisms have been used for the removal of nutrients and pollutants from a variety of waters and wastewaters over the past thirty years. Over the past decade, the use of CWSs has extended to the removal of nutrients from urban stormwater, as a more ecologically sensible management option to the traditional method of discharging stormwater into natural wetlands.

Stormwater CWSs on the Swan Coastal Plain are designed to remove phosphorus. Phosphorus is a commonly limiting nutrient affecting plant growth and the soils of the Coastal Plain have traditionally been heavily supplemented with phosphorus for urban and agricultural purposes. Despite the aims of these systems, stormwater CWSs on the Swan Coastal Plain have indicated poor phosphorus removal, typically 60-70% lower than their designed target. In contrast, natural wetlands on the Swan Coastal Plain have indicated significantly higher phosphorus removal.

Conceptual models of phosphorus removal for CWSs suggest that phosphorus is predominantly removed by the biofilm component, suggested to account for more than half the cumulative phosphorus removal in the long-term. One hypothesis proposed to account for poor phosphorus removal in CWSs on the Swan Coastal Plain has been a lack of an active biofilm component. Biofilms cover every surface of aquatic systems in a thin film, and consist of an organic matrix of algae, fungi and bacteria embedded in polysaccharides.

This study compared the biofilms of two CWSs with four physico-chemically distinct natural wetlands on the Swan Coastal Plain in order to justify or reject the proposed hypothesis. The study consisted of two distinctly separate experimental components. The first of these components aimed at quantifying the composition and biomass of biofilms, by investigating

biofilm biomass in terms of organic, inorganic and percentage organic biomass, as well as biofilm composition in terms of the algal, fungal and bacterial component percentage cover. The second component aimed at determining the rate at which biofilm can remove phosphorus from the water column by a series of controlled nutrient depletion 'batch-culture' experiments.

The results indicated that biofilms in natural wetlands on the Swan Coastal Plain were highly variable in terms of both biomass and composition. The two CWSs sampled indicated comparable biofilm biomass and composition, with the measured parameters generally falling within the ranges observed between the natural wetlands. The composition of biofilms appeared to be a reflection of the Photosynthetically active radiation (PAR) intensity at the sediment, with the biofilms in wetlands observed having high colour (low PAR intensity) being fungal/bacterial dominated, and biofilms in wetlands observed having low colour (high PAR intensity) being algal dominated. The biofilm composition of both CWSs was fungal/bacterial dominated because of high colour.

The phosphorus removal rate by biofilm appeared to be concentration dependant, with negligible phosphorus removal at low concentrations. However, at high concentrations, the phosphorus removal rates established were significantly higher than those previously published, confirming that biofilms have the potential for significant phosphorus removal from CWSs.

This research demonstrated that biofilms have the ability to remove significant quantities of phosphorus at reasonably high rates. Poor phosphorus removal of stormwater CWSs on the Swan Coastal Plain likely result from biofilm compositions poor at phosphorus removal, resulting from CWS design that fails to optimise both biofilm biomass and biofilm composition. The research results indicated that the engineering of algal-dominated biofilm composition by manipulating CWS design, as well as increasing the surface area for biofilm growth, may significantly increase phosphorus removal.

DECLARATION

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any institution of higher education; and that to the best of my knowledge and belief does not contain any material previously published or written by another person except where due reference is made in the text.

Signature

Date .....*28-04-2000*..... ...

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Stuart A. Hawkins.

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CHAPTER 1: INTRODUCTION

Stormwater constructed wetland systems (CWSs) for the removal of phosphorus from urban stormwater have become favourable management options for minimising phosphorus input into natural wetlands. Natural wetlands have been well documented as effective nutrient sinks, with stormwater CWSs functioning by optimising these nutrient removal mechanisms. However, stormwater CWSs on the Swan Coastal Plain have indicated poor phosphorus removal efficiencies. In contrast, natural wetlands on the Swan Coastal Plain have indicated high phosphorus removal.

Biofilms are organic matrices of algal, fungal and bacterial components embedded in polysaccharides that can cover every surface within a wetland as a thin film. Conceptual models of phosphorus removal in stormwater CWSs suggest that phosphorus removal by biofilms may account for more than half the cumulative phosphorus removal from a CWS. One hypothesis that has been suggested to explain poor phosphorus removal from CWSs on the Swan Coastal Plain is that the CWSs lack a sufficient and active biofilm component.

This thesis aimed at determining the role of stormwater CWS biofilms on the Swan Coastal Plain in terms of the production of biofilm biomass, and at determining the biofilm composition. The phosphorus removal rate by biofilms was also determined. These biofilm parameters were measured against biofilm biomass and composition from a range of natural wetlands, in order to determine whether the biofilms in CWSs were comparable to the biofilms found within natural systems.

1.1 CONSTRUCTED WETLAND SYSTEMS

1.1.1 Background

Constructed wetland systems (CWSs) for nutrient removal were originally developed in the 1970's for the treatment of domestic wastewater (sewage) as an alternative to chemical treatment plants (Jones, 1995; Hamilton *et al.*, 1993). Within the past three decades, the use of CWSs has been extended to include the treatment of industrial effluent (Hamilton *et al.*, 1993; Reddy & D'Angelo, 1997), the removal of pesticides and toxic chemicals (Edgehill, 1992; Alvord and Kadlec, 1996; Edgehill, 1996; Segar and Kalia, 1999), the treatment of aquaculture waste (Abeysinghe *et al.*, 1994), as mitigation for the loss of natural wetlands (Hamilton *et al.*, 1993; Water and Rivers Commission, 1997), and more recently for the treatment of urban stormwater (Waters and Rivers Commission, 1997).

The ability of natural wetlands to provide effective nutrient sinks for organic and inorganic pollutants and to absorb new nutrient loadings has been well documented (Hammer & Bastain, 1989, cited in Buchberger & Shaw, 1995; Kadlec, 1997; Lantzke, *et. al.*, 1999). Constructed wetland systems operate by optimising the nutrient removal characteristics of natural wetlands, thereby aiming to achieve higher removal rates than in natural wetlands.

1.1.2 Stormwater CWSs

The use of CWSs for nutrient removal of urban stormwater is a relatively new concept. Urban stormwater has traditionally been discharged into natural wetland ecosystems, resulting in changed nutrient loading, water quality and wetland hydrological regimes (Lane *et al.*, 1992; Braid and Lavery, 1996; Welker, 1995, cited in Water and Rivers Commission, 1998). This traditional system of stormwater management is often inconsistent with long-term conservation goals of urban wetland ecosystems. CWSs offer ecologically responsible, potentially low-cost,

and low-maintenance options for minimising urban stormwater nutrient flows into natural wetland systems (Hamilton *et al.*, 1993; Kadlec, 1997; Lantzke, *et al.*, 1999).

CWSs remove the ongoing costs of staffing and maintenance associated with chemical treatment plants, and are therefore seen as a potentially low-cost and maintenance-free nutrient removal process (Reddy & D'Angelo, 1997). CWS use is also likely to increase due to such favourable economics (Buchberger and Shaw, 1995).

The treatment of stormwater presents a significantly different challenge than wastewater treatment since stormwater has significantly lower nutrient levels and inherent high variability in inflow (Somes and Wong, 1997; Wong and Geiger, 1997). However, stormwater CWSs are thought likely to be successful given the ability of natural wetlands to act as nutrient sinks. However, nutrient removal levels from stormwater CWSs around the world have indicated high variability with phosphorus removal ranging from a 47% export to a 86% reduction (Braid and Lavery, 1996; Water and Rivers Commission, 1997).

Stormwater CWSs also have associated secondary benefits including aesthetic and recreation values for local communities, habitat creation (e.g. bird habitat) and education/research opportunities (Bowmer, 1993; Hamilton *et al.*, 1993; Reaves and Croteau-Hartman, 1994; Balla, 1994; Persson *et al.*, 1999).

1.2 STORMWATER CWSs IN WESTERN AUSTRALIA

The designs of CWSs on the Swan Coastal Plain, Western Australia, aim at reducing phosphorus, both particulate phosphorus and filterable reactive phosphorus (FRP). Filterable reactive phosphorus includes suspended colloidally bound phosphorus and dissolved phosphorus, with the latter readily bio-available and easily assimilated by wetland biota. Excessive inputs of phosphorus into natural systems may shift the biota composition structure

by promoting plant growth towards undesirable algal species. This can result in flow-on effects such as reduced concentrations of dissolved oxygen and decreased water clarity, with potentially significant, detrimental long-term effects on the biological communities and ecosystem functioning (Lee *et al.*, 1978). It is because of the bioavailability of FRP that makes it the phosphorus form of major concern for treatment in stormwater CWSs on the Swan Coastal Plain.

Magnifying this problem is that the soils of the Swan Coastal Plain are typically deficient in phosphorus (Chambers, 1984), a result of a low phosphorus retention capacity (Water and Rivers Commission, 1997). Consequently, the soils of the Swan Coastal Plain have traditionally been heavily supplemented (and continually re-supplemented) with fast-release high-phosphate fertilisers for both agricultural and domestic purposes (Chambers, 1984). The low phosphorus retention capacity results in the phosphorus supplements washing into adjoining waterways and groundwater, either by direct runoff or via stormwater drainage systems.

Limited monitoring of stormwater CWSs on the Swan Coastal Plain has prevented rigorous assessment of their effectiveness (Water and Rivers Commission, 1997). However, CWSs that have been well monitored have had low FRP removal efficiencies. Bartram Road Buffer Lakes (situated 40kms south of Perth) was developed with the primary objective of reducing influent stormwater phosphorus concentrations by 30% (Water and Rivers Commission, 1997). Phosphorus removal ranging from an 18% export to a 25% reduction of FRP, and a 47% export to a 3% reduction in total phosphorus (TP) was recorded between 1992 and 1995 (WAWA, 1994; Braid and Lavery, 1996). Russell Street CWS (situated 10km northeast of Perth) had similar phosphorus retention characteristics (Braid and Lavery, 1996), falling well short of its 50% phosphorus reduction target (Water and Rivers Commission, 1997). Hammond Road CWS (situated 40kms south of Perth) had FRP removal of 5.3% in 1998, and 11.6% in 1999 (Lund *et al.* 1999, 2000).

In contrast, some natural wetlands on the Swan Coastal Plain have indicated high phosphorus removal. The Spectacles wetland (situated 55km south of Perth) averaged TP and FRP removal from influent water at 76% and 88% respectively between 1994 and 1995 (Water and Rivers Commission, 1997). Wetlands such as The Spectacles confirm the notion that wetlands can act as effective nutrient sinks. Additionally, they indicate that TP and FRP removal rates higher than the removal targets of the CWSs are possible.

The poor performance of CWSs on the Swan Coastal Plain has been attributed to non-compliance with design specifications, particularly with significantly lower hydraulic residence time (HRT) (Braid and Lavery, 1996; Waters and Rivers Commission, 1997). Reed (1995, cited in Persson *et al.*, 1999) lists insufficient provision for water storage (ie. low HRT) and hydrodynamic control as the main factors leading to poor performance. CWSs that fail to meet their nutrient removal objectives have the potential to become long-term liabilities to the community (Persson *et al.*, 1999). Additionally, these systems may truly not be low-cost, requiring long-term careful management and fine-tuning by experienced practitioners (Bowmer, 1993).

1.3 CWS DESIGN AND CONCEPTUAL MODELS

CWSs are typically assessed by a comparison of influent and effluent water quality. This process calculates the overall effectiveness of the system, but reveals little about the internal nutrient removal processes (Hamilton *et al.*, 1993; Flood and Ashbolt, 1994). While the nutrient removal potential of CWSs is well documented, the lack of knowledge on FRP removal mechanisms has hampered their wider use (Lantzke *et al.*, 1999). Understanding of the internal wetland mechanisms has typically relied on CWS conceptual models of phosphorus removal.

Conceptual models of phosphorus removal were developed as a baseline tool for CWS design, in order to outline the major internal removal mechanisms that affect CWS performance. The DLWC (1998) conceptual model generalises the levels of phosphorus removal of soils, vegetation and sediment microbial communities over time in relation to cumulative phosphorus removal (Figure 1). Similar stormwater CWSs conceptual models have been developed by Kadlec (1997), White and Wiese (1997, cited in JDA Consultant Hydrologists, 1997), Moustafa (1997) and Lantzke, *et al.* (1999), with a similar number of models developed for wastewater CWSs (see Buchberger and Shaw, 1995).

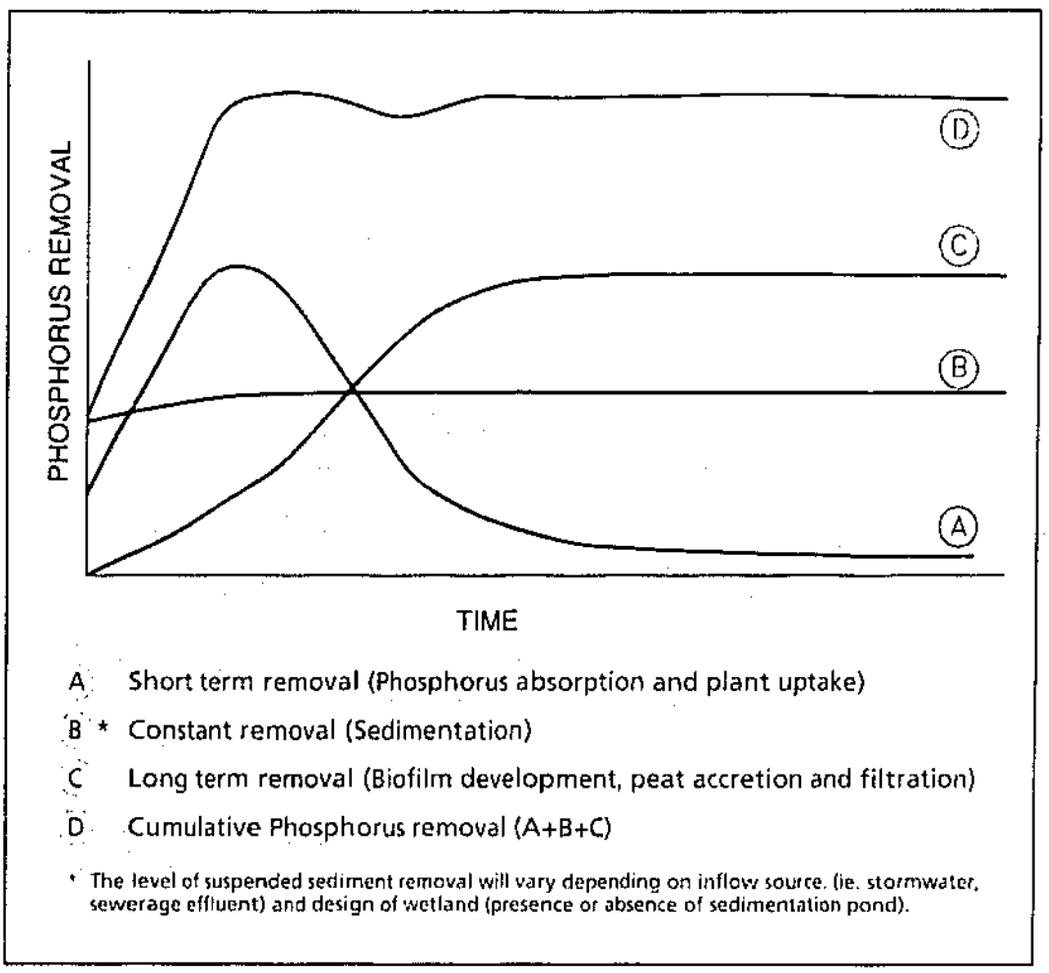


Figure 1.1 Conceptual model of phosphorus removal mechanisms in stormwater CWSs over time. Phosphorus removal levels and time scales are not indicated because levels will vary between CWSs as a result of individual environmental and design characteristics (DLWC, 1998).

Process **(A)** is the short-term removal of phosphorus by macrophyte (vegetation) uptake and absorption to the sediments. Early CWS research and development focussed on phosphorus uptake by macrophytes (Hamilton *et al.*, 1993), with the importance of both native and introduced vegetation in CWS design well documented in the literature (e.g. Lantzke *et al.*, 1993; Hamilton *et al.*, 1993; Bowmer, 1993; Tanner, 1995; Brix, 1997). However, as this more recent model suggests, phosphorus uptake by macrophytes increases rapidly in the establishment phase of the wetland, but rapidly declines and remains low when the macrophytes reach maximum biomass. Similarly, the nutrient adsorption sites of the sediments become saturated with time, reducing the role of sediments as a major nutrient sink. Beyond this point, phosphorus removal by both these mechanisms remains low. The harvesting of the above-water macrophyte biomass may promote additional macrophyte growth, thereby increasing phosphorus removal, but only in the short-term (Tanner, 1996; Brix, 1997; Lantzke, *et al.*, 1999). If the wetland vegetation is not harvested, the phosphorus bound in the macrophyte biomass may be returned back to the system by decompositional processes (Brix, 1997).

Process **(B)** is the removal of particulate bound phosphorus by sedimentation. Particulate phosphorus suspended by high-velocity stormwater flows fall to the sediment under the influence of gravity when the water velocity is reduced. The phosphorus removal rate by sedimentation remains essentially constant over the life of a wetland. The amount of phosphorus removed by sedimentation will vary according to the proportion of particulate phosphorus compared to the total phosphorus input. One of the major problems with conceptual models for CWSs on the Swan Coastal Plain is that a large proportion of total phosphorus in stormwater runoff is in dissolved or fine colloidal forms that do not settle out by sedimentation (Douglas, 1993, cited in Water and Rivers Commission, 1997). As a result of this, the anticipated role of sedimentation in phosphorus removal on the Swan Coastal Plain is diminished.

Long-term phosphorus removal is achieved by function **(C)**, the removal of phosphorus by biofilm development, peat accretion and filtration. The phosphorus removal rate of this

component is low in establishment, but increases with time to become the dominant phosphorus removal mechanism within a CWS. The conceptual model suggests that this component may account for more than half the cumulative phosphorus removal **(D)** from a CWS in the long-term. The diminished role of sedimentation on the Swan Coastal Plain further heightens the significance of these other long-term removal mechanisms.

It has been hypothesised that poor phosphorus removal in CWSs on the Swan Coastal Plain results from the absence of a significant and active biofilm component. The establishment of a viable biofilm component has been an elusive goal for CWSs designers (Duncan & Groffman, 1994), with constructed wetlands frequently having relatively low microbial activity compared to natural wetlands (Lindau & Hossner, 1981, Craft *et al.*, 1988, Langis *et al.*, 1991; cited in Duncan & Groffman, 1994). The absence of sufficient biofilm biomass would significantly constrain nutrient removal from these systems. The manipulation of constructed wetland design to promote biofilm growth and optimise biofilm activity may increase the level of FRP removed from these systems.

1.4 BIOFILMS IN CWSs

Biofilms (also known as periphyton) are a heterogeneous organic matrix consisting of algae, fungi and bacteria supported by polysaccharides that can absorb nutrients and colloids (Wetzel *et al.*, 1997; Lawrence and Breen, 1998; DLWC, 1998). Biofilms cover every surface in natural aquatic systems as a thin film (Lock, 1993, cited in Freeman *et al.*, 1995). Biofilms remove FRP by incorporating the nutrient into tissue biomass as non-reactive phosphorus (Lantzke *et al.*, 1999). Phosphorus bound within the biofilms eventually become bound within the sediment, as the biofilms become incorporated into the sediment through biofilm turnover. Biofilm turnover occurs when the existing layer is out-competed for resources by new biofilm formed above, with the existing layer becoming senescent and bound to the sediment. Complete biofilm turnover has been estimated at around ten times per year (Kadlec, 1997).

However, the factors governing the growth and activity of biofilms are not yet understood (Bryers and Characklis, 1990, cited in Flood and Ashbolt, 1994).

CWS biofilm research has largely been conducted within wastewater CWSs (e.g Edgehill, 1992; Baskaran *et al.*, 1993; Hamilton *et al.*, 1993; Flood and Ashbolt, 1994; Lindrea *et al.*, 1994; Scott *et al.*,1994; Pollard *et al.*, 1995; Alvord and Kadlec, 1996; Beyenal and Tanyloc, 1996; Edgehill, 1996; Silyn-Roberts and Lewis, 1997; Polprasert *et al.*, 1998; Rusten *et al.*, 1998; Pastorelli *et al.*, 1999), primarily because of their higher use and greater capital input for research and development. In contrast, very little biofilm research has been conducted in stormwater CWSs. The research on wastewater CWS biofilms has predominantly focussed on gross nutrient or chemical removal by biofilms or biofilm characterisation by cover, biomass or composition.

Biofilm research has also been conducted in the eastern coast of Australia in rivers and billabongs. Robertson *et al.* (1997) investigated the effect of manipulating introduced carp density on billabong biofilm biomass and macrophyte decomposition. Burns and Ryder (in press) have reviewed the potential for riverine biofilms as biological indicators of the effectiveness of management and of disturbance of riverine systems.

In stormwater CWSs, Duncan and Groffman (1994) have compared the microbial parameters from soil cores between stormwater CWSs and natural wetlands in Massachusetts and Rhode Island, USA. It was concluded that the constructed wetlands had similar microbial biomass and, in nearly all cases, the measured microbial parameters of the CWSs fell within the ranges observed in the natural wetlands. This notion was challenged by Reaves and Croteau-Hartman (1994), who stated that stormwater CWS biofilm composition can be expected to be different from natural wetland biofilms. These differences in biofilm composition would result from chemical differences of the influent water, with biofilm composition shifted to species best suited to such water chemistry. However, Reaves and Croteau-Hartman concluded that despite differences in biofilm composition, CWSs would still function similarly to natural wetlands.

Duncan and Groffman concluded that it is possible to create CWSs with a nutrient removal capacity equal to or greater than that of natural wetlands.

Mitsch *et al.* (1995) assessed phosphorus retention in a flooded riparian marsh CWS receiving stormwater in Illinois, USA. Mitsch *et al.* concluded that the biofilm and overlying water column accounted for FRP uptake of 4-6mgm^{-2}wk^{-1}. This uptake rate was lower than phosphorus removal rates by both macrophytes and sedimentation, which contrasts the conceptual model of the DLWC (1998). Cronk and Mitsch (1994) quantified biofilm biomass on natural (macrophyte) and artificial (glass) substrates in the same Illinois CWS. The study concluded that biofilms were significant users of phosphorus, contributing 1-65% of the total water column productivity at 1 to 3mgm^{-2}wk^{-1} of FRP removal. Artificial substrata accumulated less dry weight and organic weight than natural macrophyte stems, with the natural substrata showing significantly different accumulation between species. Cronk and Mitsch concluded that increasing biofilm growth would lead to increased phosphorus removal from the water column.

Burns and Ryder (in press) and Lantzke *et al.* (1999) state that knowledge of nutrient uptake kinetics of Australian biofilms is poor. Despite the significance of biofilms suggested in many CWS conceptual models, little research exists to quantify the FRP uptake rate by biofilm within these systems. Because of the lack of nutrient kinetics data, the role of biofilm within Australian CWSs remains at the conceptual stage.

Biofilm biomass and composition variability within wetlands has not been quantified for wastewater CWSs, stormwater CWSs or natural wetlands within Australia. However, given that open and vegetated habitats have distinct physico-chemical attributes, biofilms may be highly variable between habitat types. These data deficiencies may lead to inferences that biofilms are uniformly distributed within a wetland, despite clear internal wetland differences. These data deficiencies would likely be reflected in stormwater CWS design, with design failing to optimise habitat types with greatest biofilm biomass.

1.5 AIMS OF RESEARCH

An evaluation of stormwater CWSs in Perth by the Water and Rivers Commission (1997) suggested that further research should be prioritised on determining which sediment and water quality parameters affect FRP removal. This is because CWSs on the Swan Coastal Plain currently operate without sufficient knowledge of the internal mechanisms affecting their overall performance. The role of the biofilm mechanism in phosphorus removal in both stormwater CWSs and natural wetlands remains unquantified, confirming significant information gaps as to why CWSs on the Swan Coastal Plain fail to remove FRP. Knowledge of the biofilm FRP uptake kinetics remains at the conceptual stage, despite the perceived significance of biofilms in conceptual models of phosphorus removal in CWSs. This is of particular concern given that CWSs on the Swan Coastal Plain are failing to meet phosphorus reduction targets, while natural wetlands have been shown to exceed these removal levels.

CWSs on the Swan Coastal Plain may fail either as a result of poor biofilm composition, in that the algal, fungal and bacterial composition is different, or that insufficient biofilm biomass exists to remove FRP. Both of these factors, either independently or together, may result in low FRP removal. Additionally, compositional analysis may indicate whether CWSs are capable of producing similar biological communities to natural wetlands. Understanding the FRP uptake rate of biofilms may provide an insight into the FRP removal capacity of biofilms in stormwater CWSs. Additionally, the engineering of biofilm characteristics that optimise FRP removal may be gained through understanding the relationships between wetland design characteristics and biofilm biomass and composition from both stormwater CWSs and natural wetlands. The understanding of the above factors may also give a detailed indication as to why stormwater CWSs on the Swan Coastal Plain fail to remove FRP.

This research had three specific aims based on the information gaps affecting stormwater CWSs on the Swan Coastal Plain, to;

- Compare the biofilm biomass of stormwater CWSs and natural wetlands of the Swan Coastal Plain.

- Compare the biofilm composition of stormwater CWSs and natural wetlands, and
- Experimentally determine the FRP uptake potential of biofilm, in order to determine the potential contribution by biofilm to FRP removal.

To achieve the first two aims, field sampling was undertaken to collect biofilms from both open water and vegetated habitats in four natural wetlands and two stormwater CWSs over a fourteen-week sampling period. Samples collected were analysed by composition in terms of algal, fungal and bacterial cover, and for biomass composition in terms of its organic and inorganic components.

The third aim of determining the potential for biofilm contribution to FRP removal was achieved by a nutrient depletion 'batch-culture' experiment at different FRP concentrations. This experiment provided potential biofilm FRP removal rates in order to assess the potential both for biofilm to remove FRP, and for comparison of these values to the expected removal rates suggested by CWS conceptual models.

CHAPTER 2: METHODS

This chapter is divided into three distinct sections. The first section covers the wetland selection process and details of the wetlands selected. The second section covers the methodology of the field research programme used to determine biofilm biomass and composition in the natural wetlands and CWSs selected. The final section covers the methodology of the laboratory experiments used to determine the FRP uptake capacity of biofilm.

2.1 WETLAND SELECTION

2.1.1 Natural Wetland Selection

The selection of natural wetlands was based on the assumption that variability in biofilms, if variability existed, would likely be reflected by different physico-chemical characteristics. This assumption is validated by the knowledge that species have specific tolerance limits to certain physical and chemical conditions, and thus environments with different environmental conditions have specific species compositions reflective of those environments. Davis *et al.* (1993) collected physico-chemical data from 41 wetlands on the Swan Coastal Plain during January and November 1989 and November 1990. The data were analysed by agglomerative hierarchical UPGMA (unweighted pair group arithmetic averaging) classification to cluster wetland samples from all periods into groups based on physico-chemical similarity (Figure 2.1). The classification identified wetland samples that were most similar (clustered vertically) and least similar (separated vertically) by physico-chemical characteristics, irrespective of the

wetland or sample time. Four major groups were identified for further investigation from this classification.

Wetlands that had most of the individual sampling periods within a single physico-chemical grouping were identified as being consistently representative of each of the four groups. For example, Lake Balannup was sampled during November 1989 and November 1990 only and both sampling periods for the wetland occurred within Group 1. Therefore, Lake Balannup was selected as a representative of that physico-chemical group.

Where any of the four physico-chemical groups had two or more wetlands that were representative of the group, wetland selection between the groups was refined by the additional selection criteria of:

- *Vegetation:* Wetlands with a well-vegetated perimeter and/or inner vegetation were selected over wetlands in which the vegetation was severely degraded or no vegetation remained.
- *Permanence:* A range of permanent, semi-permanent and seasonal wetlands was desirable.
- *Location:* A range of wetlands that cover northern and southern wetlands (where suitable) to cater for differences in soil and sediments.

The wetlands selected for the research programme are indicated in Table 2.1. The physico-chemical grouping, the number of sampling occasions and the number of sampling periods that fell within each grouping are also shown.

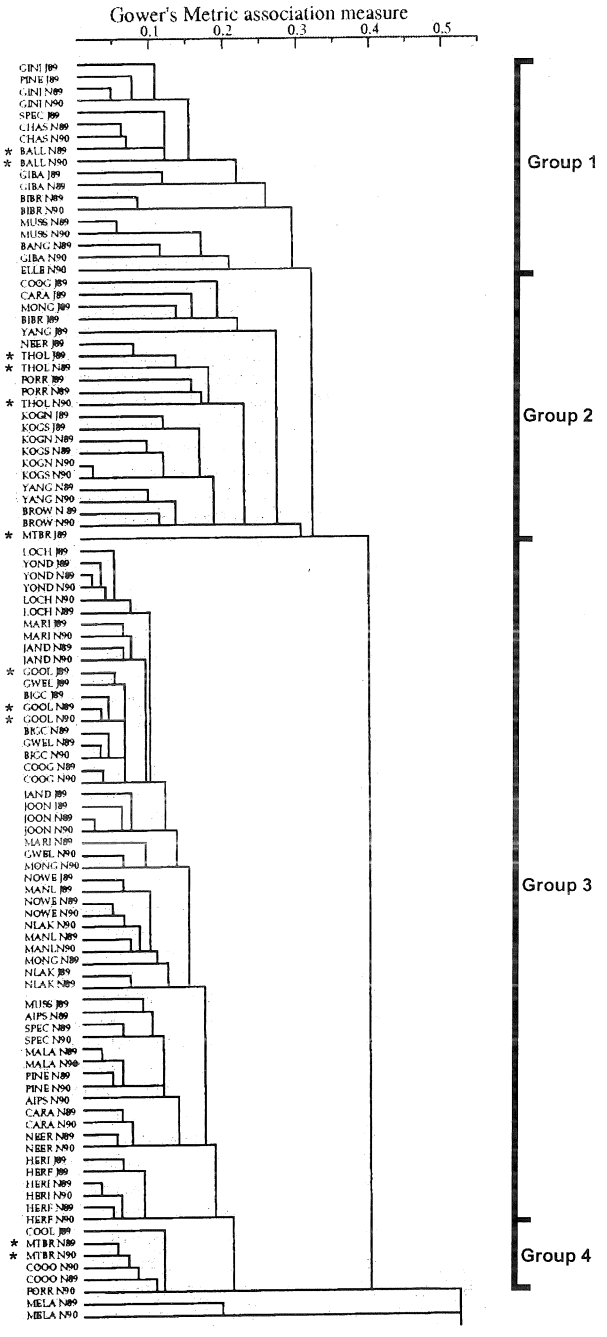


Figure 2.1 Agglomerative hierarchical UPGMA (unweighted pair group arithmetic averaging) classification of the 41 wetlands on 3 sampling occasions from Davis *et al.* (1993). The physico-chemical groupings (Groups 1 to 4) are shown to the right. The sampling periods of each of the selected wetlands are marked with an asterisk to the left.

Wetland Selected	Group 1 *Lake Balannup*	Group 2 *Lake Thomsons*	Group 3 *Lake Goollelal*	Group 4 *Lake Mount Brown*
No. Sampling Periods	2 (N89, N90)	3 (J89, N89, N90)	3 (J89, N89, N90)	3 (J89, N89, N90)
No. Sampling Periods within Group	2 (N89, N90)	3 (J89, N89, N90)	3 (J89, N89, N90)	2 (N89, N90)

Table 2.1 Wetlands selected for the research programme based on the four physico-chemical groupings identified from data from Davis *et al.* (1993). The number of sampling periods indicate the number of times the wetlands was sampled by Davis *et al.* (1993). The number of sampling periods within the group indicates the number of sampling periods that fell within the physico-chemical grouping. J89 = January 1989 sampling, N89 = November 1989 sampling, N90 = November 1990 sampling.

2.1.2 Constructed Wetland Selection

Two CWSs were selected for the research programme, Hammond Road Experimental Wetlands (Hammond Road CWS) and Bartram Road Buffer Lakes (Bartram Road CWS). Hammond Road CWS and Bartram Road CWS were both designed to remove phosphorus from urban stormwater. Both CWSs receive highly stained influent stormwater from a drain leading into Lake Thomsons, enabling physico-chemical comparability between the two CWSs, as well as comparability with Lake Thomsons, which was previously selected as one of the natural wetland for the research programme. Both CWSs selected had a known operation age, as well as known design characteristics and a documented nutrient removal history. Both CWSs selected also enabled the ability to assess whether differences in biofilm composition and/or biomass developed with the increasing age of the system, while still maintaining physico-chemical comparability between the systems.

2.1.3 Wetland Descriptions

A sitemap of the study region, and maps and photographs of each of the six selected wetlands are given in Figures 2.2 to 2.8 and Plates 2.1 to 2.6. Descriptions of each of the wetlands are also given, adapted from Davis *et al.* (1993).

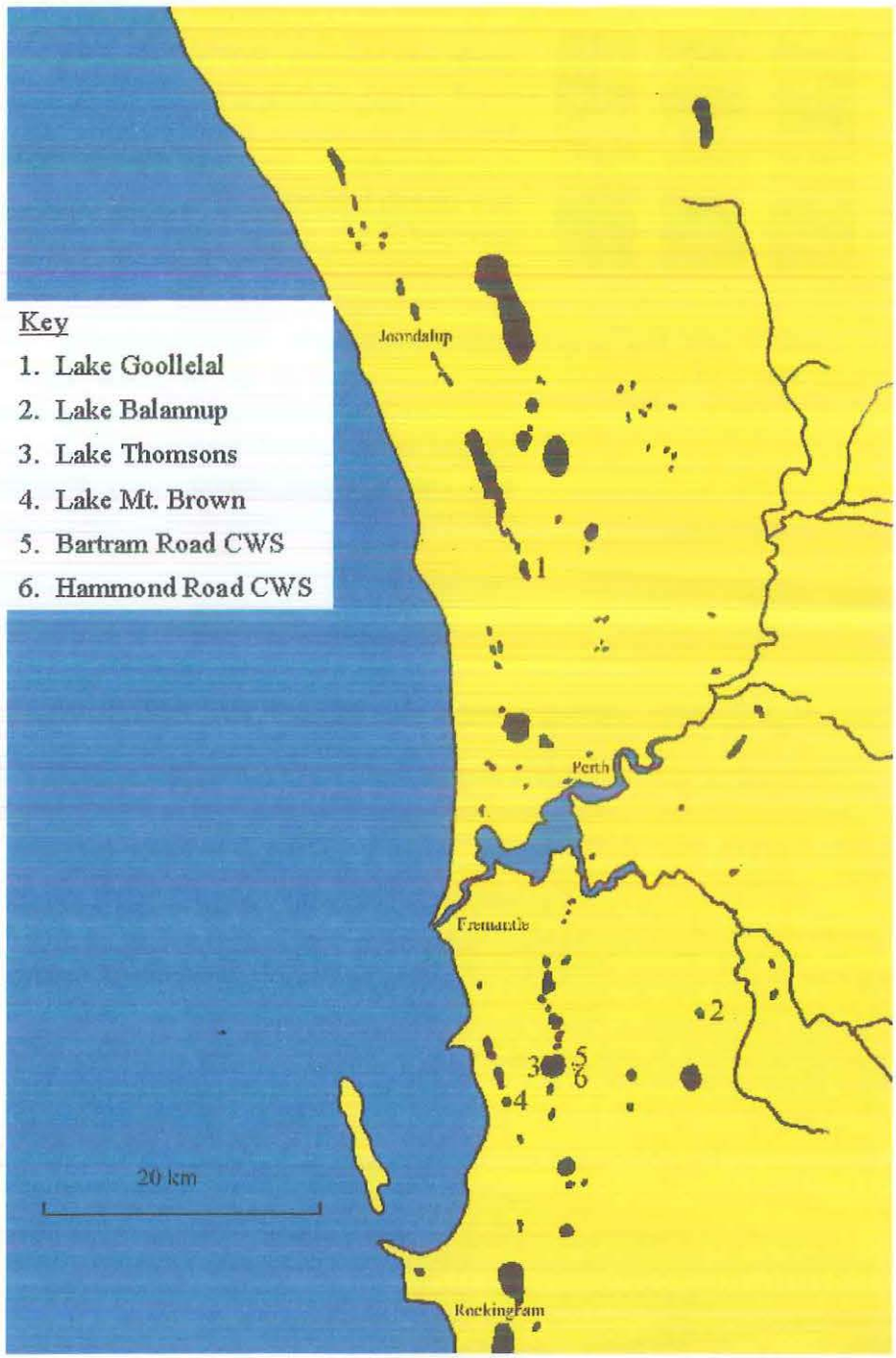


Figure 2.2 Sitemap of the Study Region. Lake Goollelal, Lake Balannup, Lake Thomsons, Lake Mount Brown, Bartram Road CWS and Hammond Road CWS are shown in relation to other natural wetlands on the Swan Coastal Plain, Western Australia. (Sitemap adapted from Davis *et al*, (1993).

2.1.3.1 Hammond Road CWS

The Hammond Road CWS consists of three separate experimental wetland cells each 15m x 5m x 1.5m (length x width x maximum depth) vegetated with *Schoenoplectus validus* in $5m^2$ sections at both cell ends. The wetland design was based upon a CWS proposed for the Ellenbrook catchment outlined by JDA Consultant Hydrologists (1997). The cells are concrete lined to prevent interaction with the groundwater, enabling water levels to be manually controlled and preventing groundwater from interfering with the known influent nutrient levels. The heavily stained influent water comes from a drain that leads into Lake Thomsons. The system commenced operation in March 1998 and has had continual monitoring since that time (Lund *et. al.*, 1990).

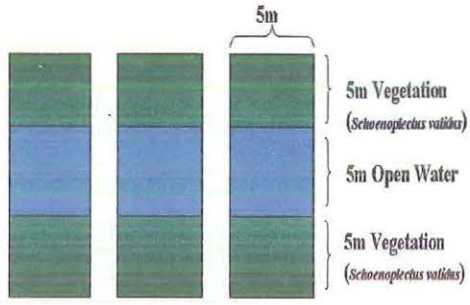


Figure 2.3 Hammond Road CWS design. Green and blue coloured areas indicate vegetated and open water habitats respectively. The cell dimensions are also shown.

Plate 2.1 Hammond Road CWS (November 1999) showing portions of Cell 2 (front) and Cell 3 (back). Vegetation of *Schoenoplectus validus* can clearly be seen. Each cell is divided by a concrete walkway (centre) by which each experimental cell can be accessed.

2.1.3.2 Bartram Road CWS

Bartram Road CWS (Bartram Road Buffer Lakes) was established in 1993 by the Water Corporation (Water Authority of Western Australia, 1993) and is a modified natural wetland (Braid & Lavery, 1996). The heavily stained influent water comes from a drain that leads into Lake Thomsons. The water level fluctuates seasonally with the groundwater level, with the lakebed drying completely during summer. The lake system consists of five varying sized lakes with well-vegetated perimeters of native and introduced species (introduced species predominantly *Typha sp.*). Influent passes through vegetated sections as it flows between each of the lakes.

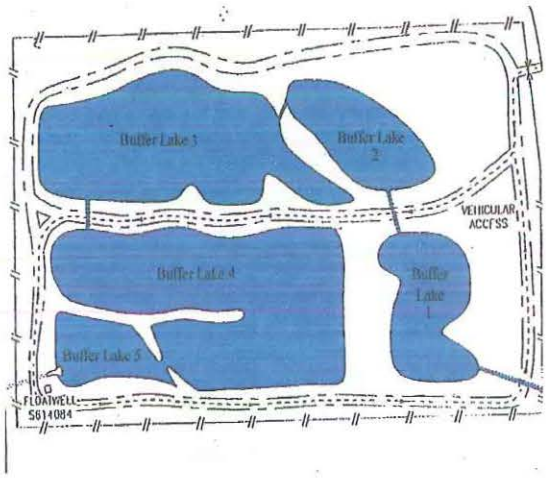


Figure 2.4 Bartram Road CWS design. Shown are the five buffer lakes (Numbered 1 to 5) and the drain that leads into Buffer Lake 1. (Map adapted from WAWA, 1993)

Plate 2.2 Bartram Road CWS buffer lake No. 2 (November 1999). The perimeter of the CWS is well vegetated with some inner vegetation also visible.

2.1.3.3 Lake Goollelal

Total Area: 60.7 ha

Open Water: 44.9 ha

Management Status: CALM Reserve

Lake Goollelal is a permanent and moderately stained nutrient enriched wetland within Yellagonga Regional Park. The Park contains a number of Perth's northern wetlands. Lake Goollelal was the deepest of all wetlands selected with a maximum depth of 1.4m. The perimeter is vegetated with a mixture of native and introduced species (introduced species predominantly *Typha sp.*). Only 16% of the fringing native vegetation remains as a result of surrounding urban and horticultural development. Inner parts of the lake are well vegetated with native rushes.

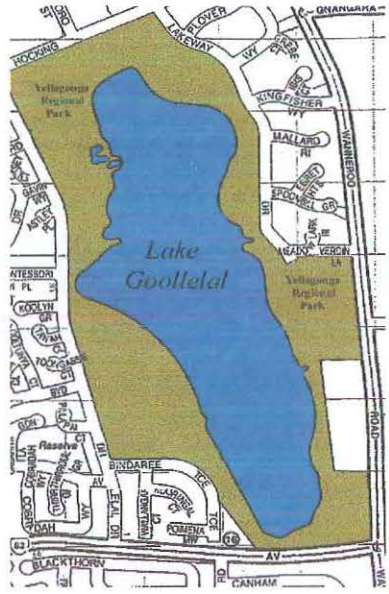


Figure 2.5 Sitemap of Lake Goollelal. The wetland is part of Yellagonga Regional Park, with residential development fringing all sides. (Map adapted from DOLA, 1994)

Plate 2.3 Lake Goollelal (November 1999). Native vegetation stands within the wetlands are clearly visible. The perimeter vegetation (foreground) consists predominantly of *Typha sp.* Residential development of the suburb of Kingsley can be seen in the background.

2.1.3.4 Lake Thomsons

Total Area: 253.7 ha

Open Water: 151.0 ha

Management Status: CALM A-Class Reserve

Lake Thomsons is a shallow and nutrient enriched wetland. Lake Thomsons is semi-permanent, in that it only occasionally dries completely in summer. The wetland is moderately coloured with 96% of perimeter vegetation remaining, consisting of native and introduced species (introduced species predominantly *Typha sp.*). Nutrient enrichment comes from the surrounding horticulture and residential areas. The benthos is dominated by a submerged aquatic grass species that covers the sediment layer throughout.

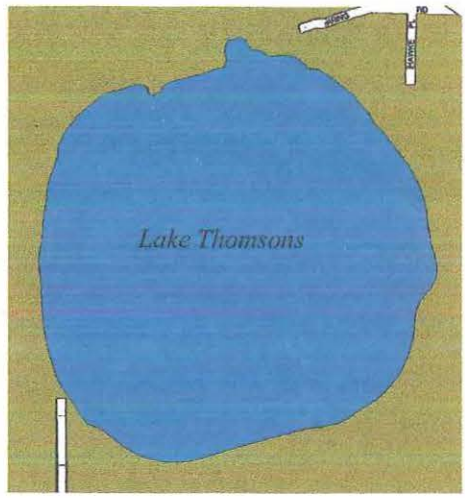


Figure 2.6 Sitemap of Lake Thomsons. Most of the area surrounding Lake Thomsons is well vegetated. However, several urban access roads pass near the lake edge. (Map adapted from DOLA, 1994)

Plate 2.4 Lake Thomsons (November 1999). The perimeter is highly vegetated with no visible residential development. During summer the open water areas dry and become inhabited by terrestrial plants.

2.1.3.5 Lake Balannup

Total Area: 20.0 ha

Open Water: 8.0 ha

Management Status: CALM Reserve

Lake Balannup is a shallow seasonal wetland with 83% of perimeter vegetation remaining. The water is heavily stained and nutrient enriched. The surrounding land use is industrial, residential and semi-rural land. Ranford Road crosses through the centre of Lake Balannup to divide the wetland into two sections. *Lemna sp.* carpeted the surface of the open water habitat during the sampling period.

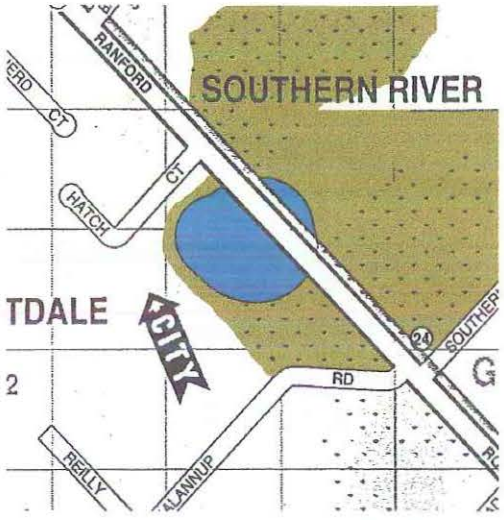


Figure 2.7 Sitemap of Lake Balannup. Ranford Road divides Lake Balannup into two sections. The smaller section was not sampled. (Map adapted from DOLA, 1994)

Plate 2.5 Lake Balannup (November 1999). The internal wetland vegetation is *Melaleuca sp.* and flooded gum in a closed canopy. Most open water sections (as shown) were blanketed by *Lemna sp.* during sampling.

2.1.3.6 Lake Mount Brown

Total Area: 15.9 ha

Open Water: 5.3 ha

Management Status:

CALM/City of Cockburn Reserve

Lake Mount Brown is a saline, shallow and seasonal wetland with moderately stained water. The perimeter vegetation of Lake Mount Brown is relatively undisturbed with 100% perimeter vegetation cover remaining. A few motor vehicle bodies and household rubbish could be found within the wetland and surrounding reserve area. A major transport route passes approximately 15m from the waters edge of one side of the wetland.

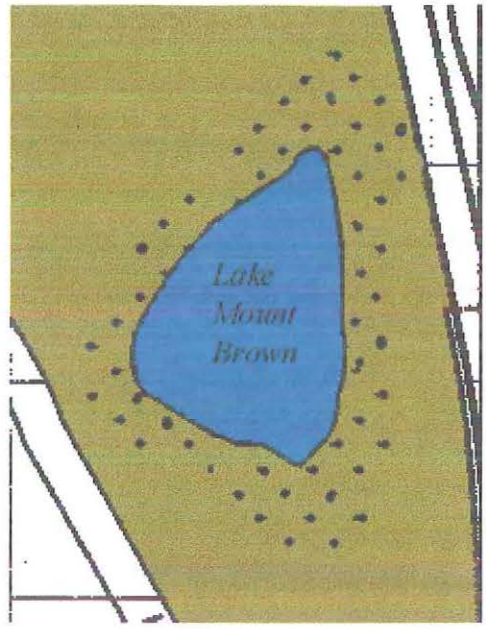


Figure 2.8 Sitemap of Lake Mount Brown. (Map adapted from DOLA, 1994)

Plate 2.6 Lake Mount Brown (November 1999). All perimeter vegetation remains, with a mixture of rush species and saltwater *Melaleuca sp.*

2.2 DETERMINING BIOFILM BIOMASS AND COMPOSITION

2.2.1 Experimental Design

The six wetlands were sampled four times over a fourteen-week period to encompass temporal variability of biofilms that may have existed in any of the selected wetlands over the period of the research project (Table 2.2). Samples were collected on large biofilm collection plates (LBCPs) for biomass and small biofilm collection plates (SBCPs) for microscopic viewing of biofilm composition (see Section 2.2.3.1). The LBCPs and SBCPs were placed in the field for a period of 14 days, as per APHA (1995).

Sampling Period	Placement Date	Retrieval Date
1	September 29, 1999*	October 13, 1999*
2	October 27, 1999	November 10, 1999
3	November 24, 1999	December 8, 1999
4	December 22, 1999**	January 5, 2000**

Table 2.2 Placement and retrieval dates for each sampling period. * Lake Balannup not sampled due to access restrictions. ** Lake Balannup and Lake Thomsons were not sampled as the wetlands were dry.

In each wetland, open water and vegetated habitats were sampled (Figure 2.9). Three randomly located samples were collected from both open water and vegetated habitats in each wetland. Hammond Road CWSs was the only exception to this, with samples located randomly within each habitat type in each of the three experimental wetland cells. The samples were retrieved after 14 days and returned to the laboratory for biomass and composition analysis. Biomass analysis involved calculating the organic biomass, inorganic biomass and percentage organic biomass of the biofilms. Compositional analysis involved calculating the algal, fungal and bacterial components of the biofilms by percentage cover and analysis of the chlorophyll *a* biomass. Chlorophyll *a* biomass, a measure of algal biomass, was

sampled as a single sample only, in order to compliment the algal percentage cover data. A single Chlorophyll *a* sample was taken from biofilms from each habitat for each wetland on each sampling occasion. The analytical techniques are outlined in Section 2.2.3.

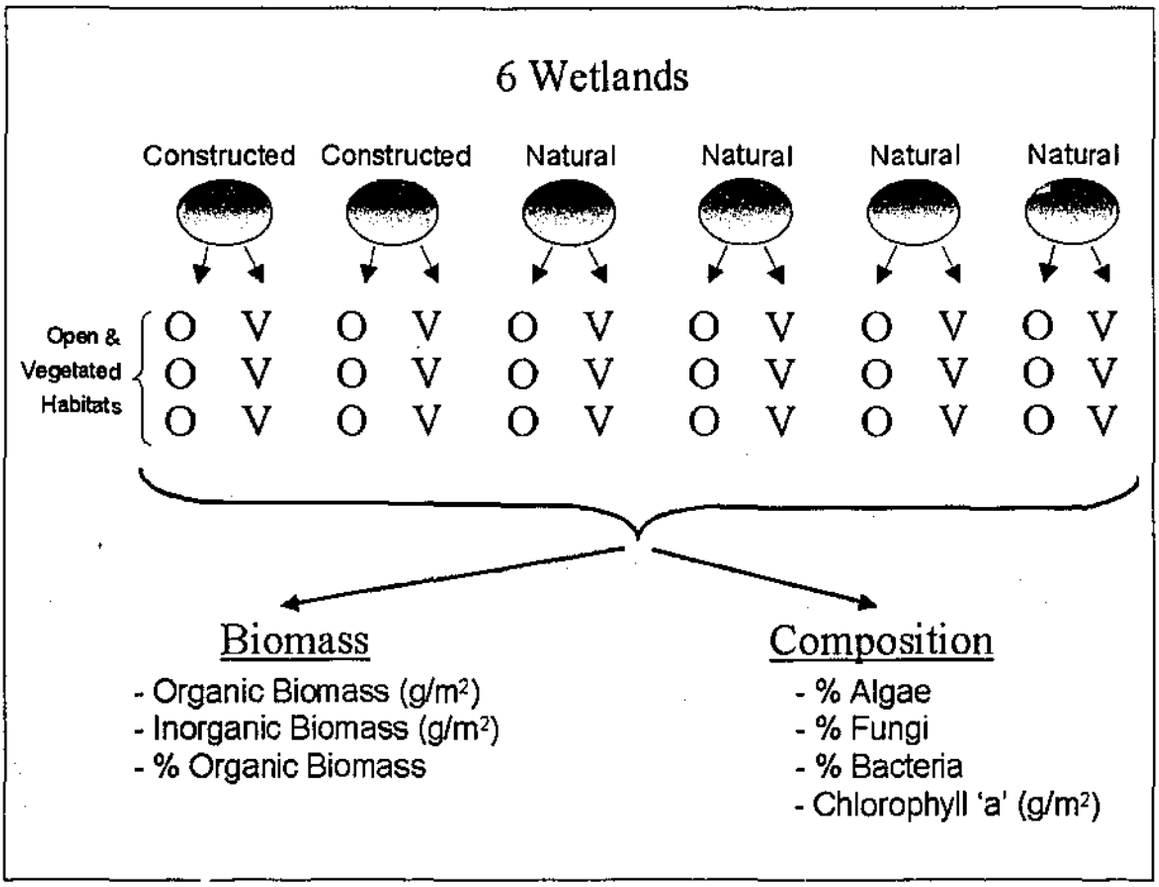


Figure 2.9 Experimental Design for each of the four sampling periods. Biomass and composition data was collected from each habitat of the six wetlands selected

2.2.2 Statistical Design, Analysis and Transformations

The sampling design permitted a 2-factor Analysis of Variance (ANOVA) with the fixed factors of 'wetland' and 'habitat'. It was hypothesised *a priori* that time was not a significant factor. Multivariate analysis was conducted to determine whether there was temporal

variability in biofilm biomass and biofilm composition. A similarity of samples was first produced using the Bray-Curtis similarity measure by the 'Cluster' module in the PRIMER (Plymouth Routines in Multivariate Ecological Research) statistical software package (Plymouth Marine Laboratory, 1994). The Bray-Curtis similarity measure was performed on transformed biomass data (organic and inorganic biomass transformed to a 0-100 scale) and un-transformed percentage cover data (algal, fungal and bacterial percentage cover) from all wetlands.

Ordination by multi-dimensional scaling (MDS) based on the Bray-Curtis similarity measure was conducted and plotted with the aid of the Deltagraph™ graphics package (SPSS Inc., 1997). Missing values in the data set were deleted in order to conduct the MDS. The MDS provided a 2-dimensional visual representation of how similar samples were to each other, with samples split by wetland, habitat and time. Samples that were most similar clustered together, while samples that were least similar were further apart.

The lack of temporal clustering between and within wetlands in the MDS indicated that no temporal variability in biofilms existed. As a result of this, data from the different sampling periods for each wetland were pooled within habitats, so that the number of habitat replicates increased from 3 replicates to 12 replicates (3 replicates x 4 sampling periods). A 2-factor Analysis of Variance (ANOVA) was conducted using the SPSS V10.0 statistical package (SPSS Inc., 1999) to determine whether significant differences existed between wetlands and/or between habitats. Tukey-HSD Post-hoc testing was conducted simultaneously in order to determine where significant differences occurred between wetlands. Both ANOVA and Tukey-HSD required normally distributed data. Data in ratio and percentage form were transformed using the arcsine transformation to convert the data from a binomial distribution to a normal distribution (Zar, 1996). All data (including arcsine transformed data) were tested for normality by Levene's Test of Equality of Error Variances. Data not conforming to a normal distribution were transformed by using Square Root and Log-10 transformations as per Zar (1996).

2.2.3 General Analytical Techniques

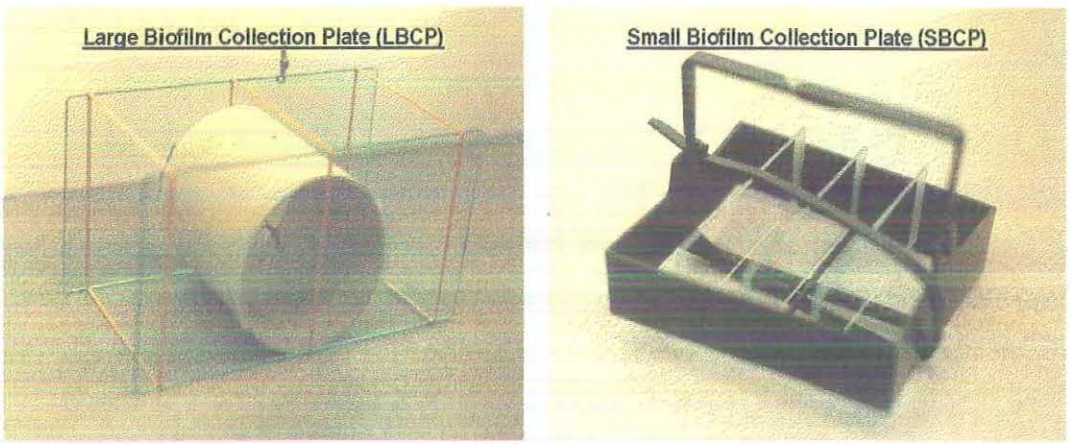
2.2.3.1 Wetland Collection of Biofilm

Biofilms from each wetland were collected on glass plates over a 14 day period, as per APHA (1995) Section 10300 B. Two types of glass plate structures were used.

Large biofilm collection plates (LBCPs) were used for the collection of biofilm biomass, chlorophyll *a* biomass and for FRP uptake kinetic experiments. This large surface area of glass was required to provide sufficient biofilm biomass for sampling. Each LBCPs consisted of two large panes 200mm x 100mm x 2mm (length x width x thickness) fastened to either side of a 1000mm x 900mm circular PVC divider by rubber bands (Plate 2.7a). The divider had holes in the bottom to allow for water drainage. Where the divider was in contact with the glass, one third of the diameter of the divider was secured to each pane with silicon. After securing with silicon, the plates were oven-dried for two hours at 50^0C to ensure that the silicon cured. Silicon releases acetic acid during the curing process, and so the plates were then bathed in deionised water for a minimum of three hours to remove acetic acid, any glass fragments and PVC dust. New glass panes were used for each round of sampling. The PVC dividers were reused for successional sampling periods, after cleaning.

Small biofilm collection plates (SBCPs) were used for the collection of biofilm for compositional analysis. Each SBCP consisted of three standard glass microscope slides 75mm x 25mm x 1mm (length x width x thickness) placed in an open microscope slide rack fastened with a cable tie (Plate 2.7b). Slides were arranged with maximum distance between each slide to allow water circulation between the slides. Microscope slides were used to enable microscopic viewing of the biofilm structure.

Both types of plates were designed so that the glass panes were positioned vertically in the water column to minimise any sedimentation on the glass that could smother the biofilm matrix (APHA, 1995). The plates were gently lowered through the water column in each wetland to be rested on the surface of the sediment. White fishing floats were fastened to each sampling unit (consisting of one LBCP and one SBCP) to allow for the relocation of samples in the field.



a) b)

Plate 2.7 The LBCP and SBCP designs. The glass panes were positioned vertically to minimise potential sedimentation problems. Both structures were gently lowered to rest on the surface of the sediment

2.2.3.2 Analysis of Biomass

2.2.3.2.1 Biofilm Biomass

Biofilm dry weight, organic biomass and inorganic biomass was conducted as per APHA (1995) Section 10300 C. Biofilm was removed from both external sides of each LBCP using a razor blade and transferred to a clean 10ml centrifuge tube. Samples were frozen at 0°C until they could be processed. For processing, samples were transferred to pre-fired, pre-weighed crucibles, dried at 105°C and

weighed to record the dry weight. Samples were then ignited at 550°C for two hours. Water-of-hydration was reinstated before the samples were weighed to determine the inorganic biomass. The organic biomass was calculated as the difference between dry weight and the inorganic biomass. The percentage organic composition was calculated as the organic biomass percentage of the total dry weight.

2.2.3.2.2 Chlorophyll *a* Biomass

Chlorophyll *a* was calculated as per Speziale *et al.* (1984). Biofilm samples were obtained by scraping the two outside surfaces of one LBCP into a clean 10ml centrifuge tube. Chlorophyll *a* was extracted using 10ml N,N-Dimethylformamide (DMF). Samples were refrigerated for 24 hours and then centrifuged at 3000rpm for 3 minutes before removing the extractant. The optical density of the extract was measured at OD665 and OD750, acidified with 0.2ml of 0.1M HCl and measured at OD665 and OD750 ninety seconds after acidification. Four-centimeter path-length glass cuvettes were used during analysis to increase the accuracy of the optical density measurements (APHA, 1995). Chlorophyll *a* was calculated as:

$$\text{Chl } a \ (\mu gm^{-2}) = \frac{11.7 \times 2.43 \times (665b - 665a) \times V}{A \times l}$$

Where:
- 665a = absorbance at OD665 after acidification
- 665b = absorbance at OD665 before acidification
- V = total volume (ml) of solvent used for extraction
- A = surface area of biofilm (m^2)
- l = path-length of the cuvette

2.2.3.3 Analysis of Composition

2.2.3.3.1 Slide Preparation

The SBCPs were dismantled in the laboratory and the slides removed from each slide rack. The slides were labeled with wetland, habitat and location details. Two slides from each SBCP were fixed with 5% glutaraldehyde in a phosphate buffer and then stained with 0.1% acridine orange as per APHA (1995) Section 9216 B. The remaining slide was prepared by fixing in 5% formalin as per APHA (1995) Section 10300 B (3). Cover slips were permanently fixed to all slides by clear nail varnish to protect the biofilm structure.

2.2.3.3.2 Algal and Fungal Percentage Cover

Algal and Fungal percentage cover was determined using the point intercept method as per APHA (1995) Section 10200 F, under oil using x1000 magnification. A 10x10 grid Whipple disk was placed in the ocular lens and used as the reference. The area covered by the grid was measured at 25μm^2 using a stage micrometer.

One microscope slide from each SBCP was selected at random and the percentage cover of algae and fungi from three fields of view recorded. The mean cover for algae and fungi from the three fields of view was recorded as the percentage cover. Percentage cover was expressed as both absolute percentage cover and as a relative cover of each taxonomic type to the total biofilm cover.

2.2.3.3.3 Bacterial Percentage Cover

Bacterial percentage composition could not be calculated by the intercept method, as the bacterial cells were significantly smaller than the viewing grid cells. Using the intercept method would therefore have resulted in a gross overestimation or underestimation of the true bacteria percentage cover.

Instead, the number of individual bacterial cells were counted from the grid area of photographs taken of each field of view used in the algal and fungal percentage cover analysis. The percentage cover of bacterial cells in the grid was expressed as a percentage of the number of bacterial cells that would be required to fill the entire grid. The mean bacteria percentage cover from the three fields of view was recorded as the bacterial percentage cover. Percentage cover was expressed as both absolute percentage cover and as a relative cover of each taxonomic type to the total biofilm cover

2.3 DETERMINING BIOFILM FRP UPTAKE

2.3.1 Experimental Design

The uptake of FRP by biofilm was determined by a nutrient depletion batch-culture experiment. In nutrient depletion batch-culture experiments the uptake of FRP is calculated as the loss of FRP from the surrounding solution. The biofilm uptake of FRP was determined by measuring the depletion of FRP from solution at five concentrations: $<50\mu gL^{-1}$, $50\mu gL^{-1}$, $100\mu gL^{-1}$, $200\mu gL^{-1}$ and $400\mu gL^{-1}$, with five replicates at each concentration (Figure 2.10). The concentrations chosen were selected to encompass the FRP concentrations found in wetlands on the Swan Coastal Plain (Davis *et al.*, 1993), with the highest concentration indicative of a highly enriched wetland. Water samples were taken at 0, 5, 10, 20, 35, 55, 85 and 120 minutes as determined by a pilot study of biofilm FRP uptake (Appendix B). The pilot study was conducted by measuring FRP uptake by biofilm in one tank with an FRP concentration of $100\mu gL^{-1}$. The rate of FRP uptake at each concentration was calculated as the mean slope of regression for each individual tank. The uptake rate for each concentration was normalised to the mean biofilm organic biomass at each concentration.

2.3.2 Criteria for Determining Significant Uptake

The degree of accuracy in the measurement of the FRP concentration was $\pm 3 \mu g L^{-1}$. Therefore, a change in FRP concentration greater than or equal to $3 \mu g L^{-1}$ over the sampling period was set as the criteria to determine if a measurable uptake had occurred. Changes less than $\pm 3 \mu g L^{-1}$ were determined to be within the range of analytical error.

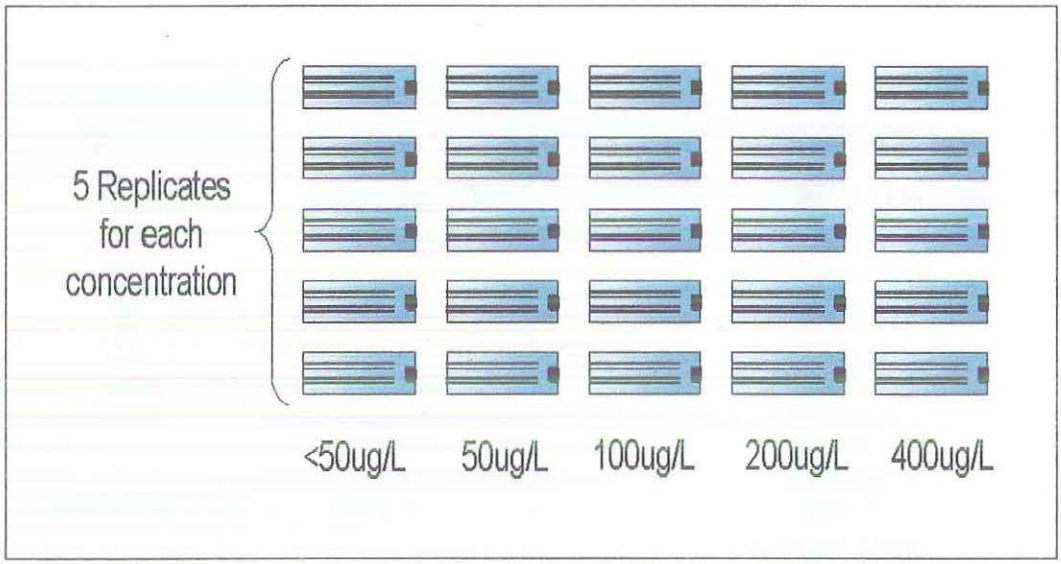


Figure 2.10 Biofilm FRP Uptake Kinetic Experimental Design.

2.3.3 FRP Uptake Tank Design

FRP uptake experiments were performed in glass chromatography tanks (Plate 2.8). Each tank held 4 LBCP panes separated in pairs by plastic dividers. The plates were held to the dividers by elastic bands. A water pump circulated the medium evenly around the plates to prevent boundary layer formation. Dye tests confirmed that the tanks were fully mixed within 5 seconds (Appendix E).

The FRP concentrations were obtained by adding the appropriate volumes of working solution and topped with filtered wetland water to a total solution volume of 2000mL. The wetland water was obtained from Loch McNess South (in Yanchep National Park) because of consistently low FRP concentrations at ~2μgL^{-1} (Sommer and Horwitz, 1999). The wetland water was filtered through 0.45μm GFC filter paper to remove particulate matter. The tanks at the <50μgL^{-1} concentration did not contain the working solution, and therefore the FRP concentration in each tank were the same as the filtered wetland water.

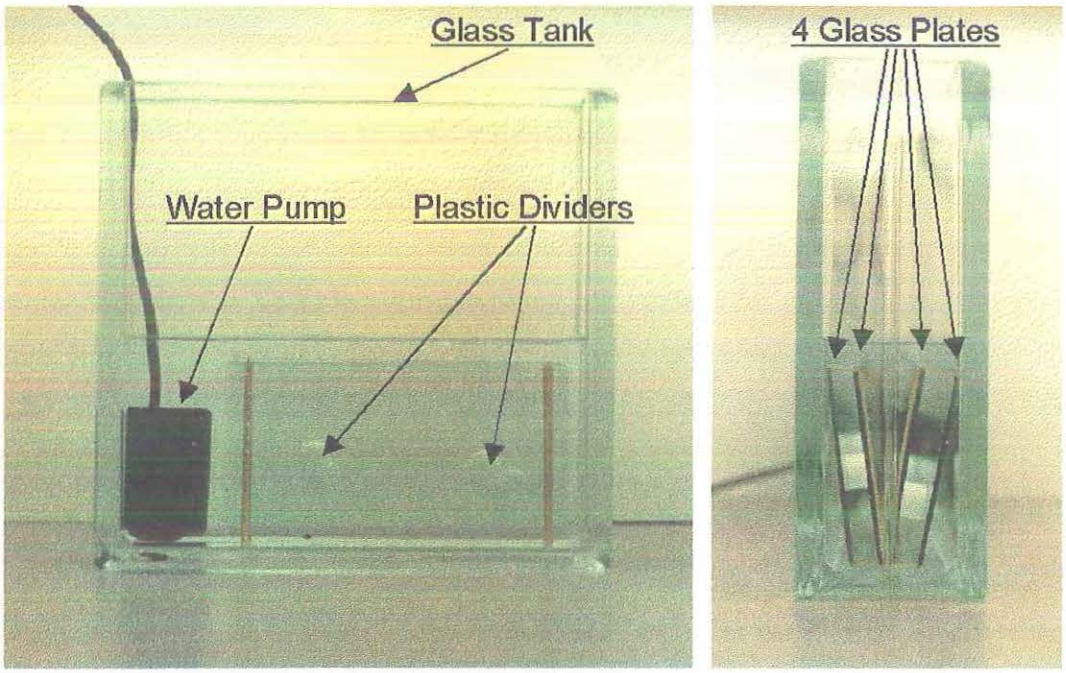


Plate 2.8 FRP Uptake Tank Design. Side and end views of the uptake tanks are shown to show the water pump and the division between plates that helped water circulation around and between the plates.

2.3.4 Wetland Collection of Biofilm

The biofilm used for the FRP uptake experiments was collected from the open water zone of the Hammond Road CWSs as it was known that it would contain water during January of 1999, which was when the FRP uptake experiments were scheduled. Biofilm was obtained from the open water habitat only, so that if significant differences in biofilm composition between the two habitat types were identified, the biofilm composition for the FRP uptake experiments would be consistent. Samples were collected on plates deployed for a 6-week period, so as to ensure that a mature biofilm standing crop (containing live and senescent biofilm components) had developed.

2.3.5 Controlled Factors in Uptake Experiments

Light and nutrient concentrations were controlled so as to simulate light conditions in the Hammond Road CWS and to ensure that nutrients, other than phosphorus, were not limiting. Photosynthetically active radiation (PAR) intensity was provided at 3.4$\mu molm^{-2}sec^{-1}$. For operator safety reasons, this was as close as practicable to the 0.2$\mu molm^{-2}sec^{-1}$ PAR intensity measured in Hammond Road CWS.

Bold's Basal Medium (BBM) (Bischoff and Bold, 1963, cited in Bold and Wynne, 1978) was added as a nutrient supplement to ensure that the biofilm was not limited by any other nutrient. The appropriate volumes of each of the seven BBM component solutions were added to each tank prior to the plates being inserted. Sodium chloride was excluded from the medium to prevent the tanks from becoming brackish. Phosphorus was also excluded from the BBM component solutions, as this was the experimental nutrient.

2.3.6 General Analytical Techniques

2.3.6.1 Glassware Preparation

All glassware and plastics used for FRP analysis were cleaned in 10% HCl for at least 3 hours, and then rinsed thoroughly with deionised water. Glassware and plastics were oven dried at 50°C.

2.3.6.2 Analysis of FRP Uptake

2.3.6.2.1 FRP Stock and Working Solution

Dipotassium hydrogen-orthophosphate (K_2HPO_4) and Potassium dihydrogen-orthophosphate (KH_2PO_4) were used for making the FRP stock solution as per Biscchoff and Bold (1963, cited in Bold and Wynne, 1978), Polprasert *et al.* (1998) and Pastorelli *et al.* (1999). The relative proportions of each orthophosphate type were based on Biscchoff and Bold (1963, cited in Bold and Wynne, 1978). 4.6007g of K_2HPO_4 and 10.7352g of KH_2PO_4 were dissolved in 1 litre of deionised water to make a $10gL^{-1}$ FRP stock solution. A working solution was formed by a 100-fold dilution of the stock solution.

2.3.6.2.2 Measuring Biofilm FRP Uptake

Ten millilitre water samples were taken from each tank using a syringe at regular intervals. Eight millilitres of the sample was filtered through a 0.45μm GFC filter paper into a clean centrifuge tube.

The FRP concentration of the filtered samples were determined by the Ascorbic Acid Method as per APHA (1995) Section 4500-P E. Ammonium molybdate and

potassium antimonyl tartrate react in an acid medium with FRP to form a phosphomolybdic acid that is reduced to an intensely coloured molybdenum blue by ascorbic acid. The FRP concentration was measured as the spectrophotomic optical density at 880nm and compared to a FRP standard curve. Higher FRP concentrations are recorded with increasing intensity of the molybdenum blue. Four-centimeter path-length cuvettes were used to increase the accuracy at which the FRP concentration could be determined from $10\mu gL^{-1}$ to within $3\mu gL^{-1}$ (APHA, 1995).

2.3.6.2.3 Analysis of Biofilm Biomass.

Biofilm biomass from plates used in the biofilm FRP uptake experiments were determined as outlined in Section 2.2.3.2.1.

2.3.6.3 Additional FRP Uptake Tests

Two additional FRP uptake experiments were performed to test the effects of PAR and BBM on the biofilm FRP uptake rate. It was hypothesised that the PAR intensity or BBM may have influenced the results obtained from the FRP uptake experiment.

Firstly, FRP uptake was determined at a higher PAR intensity and an FRP concentration of $200\mu gL^{-1}$ with 5 replicate samples. The PAR intensity was provided at $10.3\mu molm^{-2}sec^{-1}$. In comparison to the full uptake experiment, all sample extraction time remained the same except for an additional extraction at 180 minutes. The FRP uptake was measured as stated in Section 2.3.6.2.2. The biofilm from each tank was removed and analysed for organic biomass as per Section 2.2.3.2.1. The LBCPs used had been in the Hammond Road CWS for a period of 4 weeks.

Secondly, the effects of the BBM solutions on biofilm FRP uptake were tested. Limited time and a lack of biofilm plates in Hammond Road CWS did not allow for experimental

replication. The BBM uptake experiment was conducted using two uptake tanks at an FRP concentration of 250μgL^{-1}. Only one tank contained the BBM component solutions. The experiment was conducted over a 42-hour period with samples extracted at 0 hours and 42 hours. The FRP concentration was measured as stated in Section 2.3.6.2.2. The PAR intensity was provided at 10.3$\mu molm^{-2}sec^{-1}$. The plates used had been in the Hammond Road CWS for a period of 4.3 weeks.

CHAPTER 3: RESULTS

3.1 MDS ANALYSIS OF TEMPORAL VARIABILITY

The MDS results indicated that there was no temporal variation in biofilms over the research period. Temporal variation would have been indicated by the MDS through the clustering of wetlands and/or the clustering of sampling periods. The absence of definitive clustering of the sampling periods between wetlands indicated an absence of temporal variability (Figure 3.1). Additionally, there was an absence of clustering of the sampling periods within wetlands (Figure 3.2).

The MDS of all sampling periods from all wetlands confirmed high biofilm variability between wetlands. Despite a large proportion of sampling periods clustering from most wetlands (indicated by the oval), a large proportion of the sampling periods from all wetlands were dispersed outside of the cluster. This indicated that a large proportion of biofilms had high similarity and clustered together, but with an almost equal degree of high biofilm variability amongst the whole sample group that were dispersed.

Within the main cluster, Bartram Road CWS (Figure 3.2b), Lake Mt Brown (Figure 3.2c) and Lake Goollelal (Figure 3.2d) featured prominently, with a large proportion of the wetland samples in the main cluster. Bartram Road CWS and Lake Mt Brown also indicated the tightest clustering of all wetlands, indicating low biofilm internal variability between each wetland and habitat. Within the main cluster, there was no evidence of patterning on the basis of sampling time.

High biofilm variability was evident in Hammond Road CWS (Figure 3.2a), Lake Goollelal (Figure 3.2d), Lake Thomsons (Figure 3.2e) and Lake Balannup (Figure 3.2f). The high spread of samples in the MDS indicated high variability within each wetland. No patterning based on sampling time was evident in any of the wetlands. Lake Goollelal indicated high variability of sampling periods 3 and 4, with relatively low variability of sampling periods 1 and 2, which feature predominantly in the main cluster. Despite this, there was still no clustering visible based on sampling time within Lake Goollelal.

The MDS, through a lack of clustering of the sampling periods and wetlands, indicated that the time of sampling during the research period was not a significant factor. As a result of this, further analysis of the data pooled all sampling periods for each wetland.

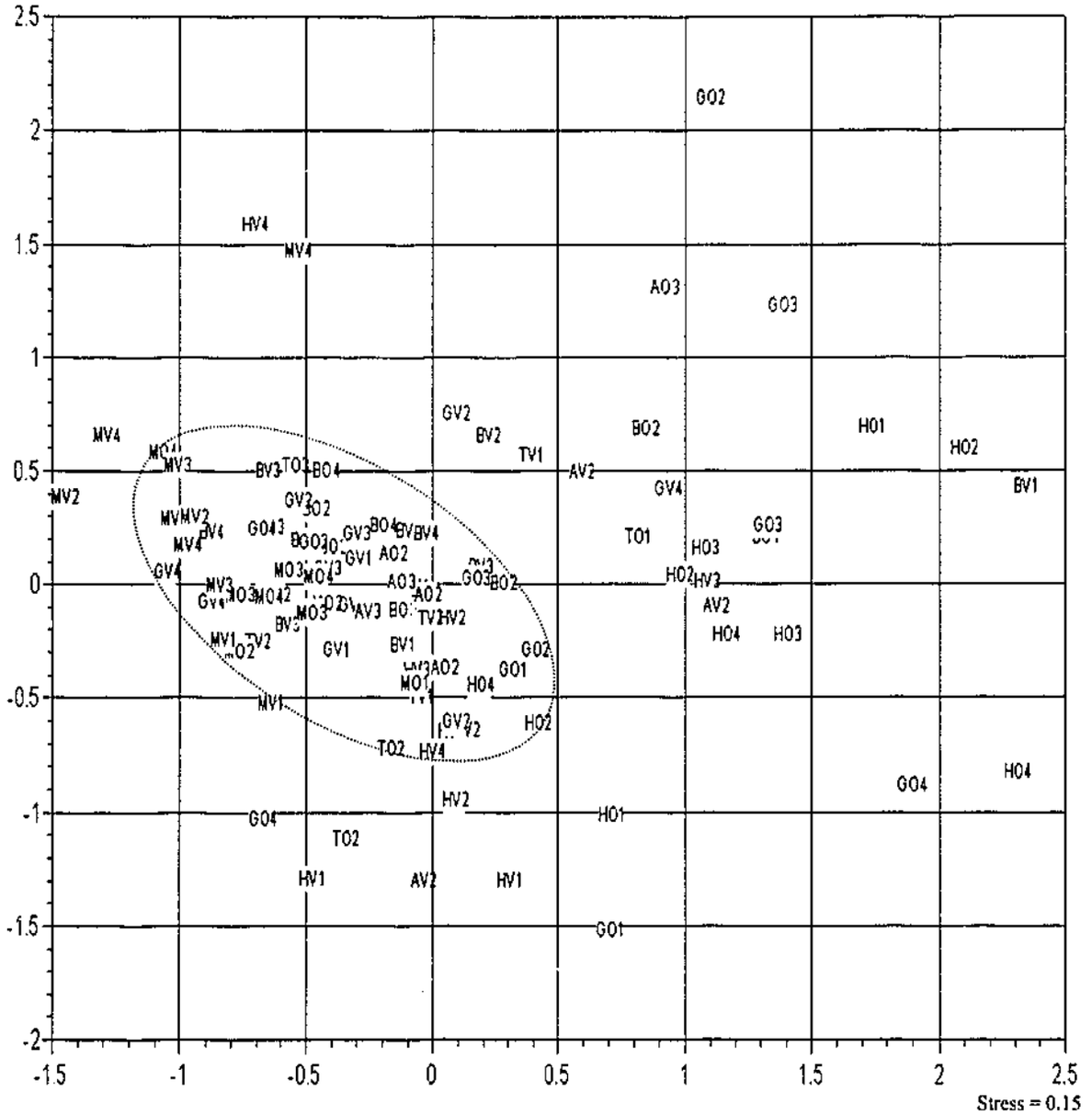
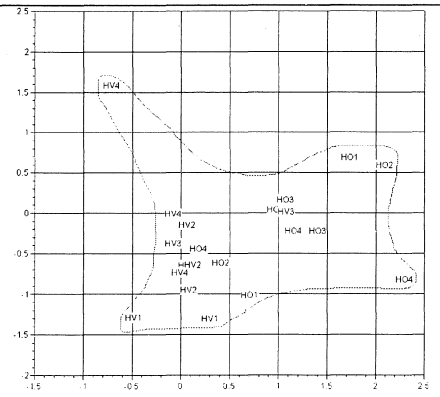
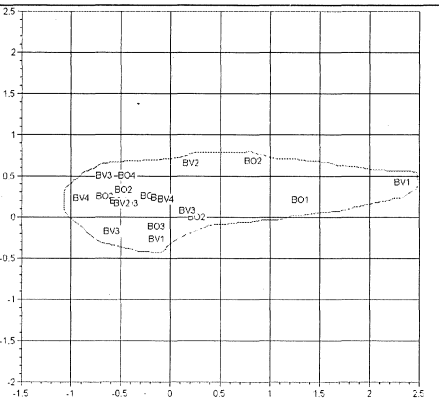


Figure 3.1 MDS ordination for all wetlands and sampling periods. No clustering was evident based on time. The input data for the MDS were Algae, Fungi and Bacteria untransformed percentage cover (absolute) and Organic and Inorganic Biomass transformed data.

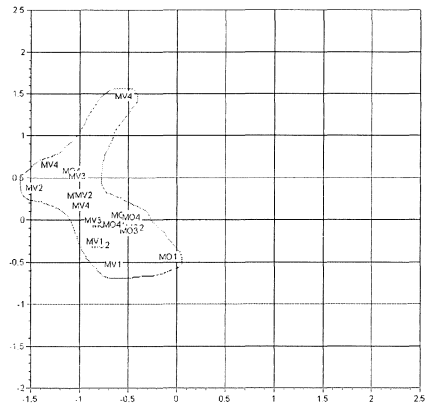
Key: Hammond Road CWS = H, Bartram Road CWS = B, Lake Goollelal = G, Lake Mt Brown = M, Lake Thomsons = T, Lake Balannup = A, Open Habitat = O, Vegetated Habitat = V. The number at the end represents the sampling period.



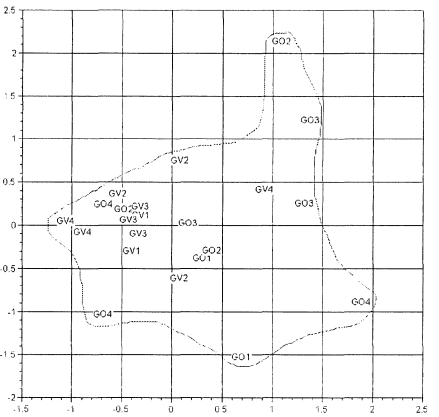
a) Hammond Road CWS



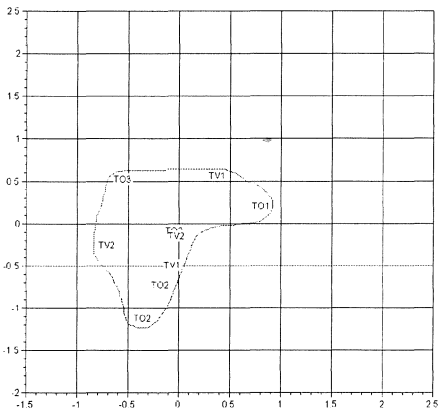
b) Bartram Road CWS



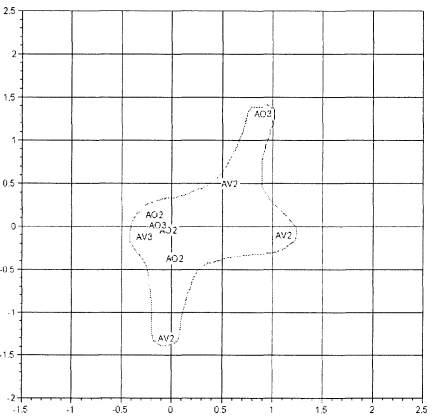
c) Lake Mt. Brown



d) Lake Goollelal



e) Lake Thomsons



f) Lake Balannup

Stress = 0.15

Figure 3.2 MDS ordination results for each wetland. The results are taken from the Figure 3.1, with each wetland separated to show the clustering pattern of sampling periods in each wetland. Lake Mt Brown and Bartram Road CWS indicated weak clustering of all sampling periods, but no clustering of the sampling periods based on time.

3.2 BIOMASS AND COMPOSITION ANALYSIS

Statistically significant differences in biofilms, both in biomass and composition, were identified between wetlands and between wetland habitat types. This confirmed high biofilm variability of biofilms on the Swan Coastal Plain.

3.2.1 Biomass

Lake Mt Brown separated from most wetlands in having the highest organic and inorganic biomass of all wetlands in both habitat types. The highest mean organic biofilm biomass for any wetland habitat was in the Lake Mt Brown vegetated habitat at $0.114gm^{-2}$, with all other wetland habitats having less than $0.05gm^{-2}$ (Figure 3.3). The organic biomass was significantly different between wetlands (0.000, 5df, $p<0.05$), with post-hoc testing confirming that Lake Mt. Brown was significantly different from all other wetlands . Lake Mt Brown also had the highest inorganic biomass for both open and vegetated habitats (Figure 3.4). Other statistically significant differences in organic biomass indicated by post-hoc testing were identified between Hammond Road CWS and Bartram Road CWS (0.003, 5df, $p<0.05$), and between Hammond Road CWS and Lake Goollelal (0.009, 5df, $p<0.05$).

Vegetated habitats had significantly higher organic biomass in all wetlands except for Lake Balannup, which had the lowest organic biomass of all vegetated habitats (0.031, 1df, $p<0.05$). The vegetated habitat of Lake Balannup also had lower inorganic biomass.

The mean inorganic biomass of biofilm was significantly different between wetlands (0.001, 5df, $p<0.005$), but not between habitat types (0.096, 1df, $p>0.05$). Lake Mt. Brown had the highest mean inorganic biomass, followed by Hammond Road CWS and Bartram Road CWS. Post-hoc testing revealed once again that Lake Mt. Brown was different from the others, being significantly different from all wetlands except for Bartram Road CWS (0.198, 5df, $p>0.05$), while Bartram Road CWS was not significantly different from any other wetland (5df, $p>.005$).

Despite clear differences of organic and inorganic biomass between wetlands and between habitat types, the biofilm organic proportions were not significantly different between wetlands (0.057, 5df, $p>0.05$) or between habitat types (0.096, 1df, $p>0.05$). This means that irrelevant of the wetland or habitat type, the organic percentage of biofilm would not be statistically different.

The organic and inorganic biomass of both habitat types for the Bartram Road CWS fell within the ranges found in the natural wetlands. Hammond Road CWS had the lowest organic biomass of all open habitats, and also the lowest organic biomass overall. The inorganic biomass of Hammond Road CWS fell within the ranges observed between the natural wetlands. The Hammond Road CWS vegetated habitat had the second highest inorganic biomass. However, it should also be noted that the standard error of this result is large.

Biofilm grazing by gastropods was observed on ten sampling plates during sampling period 2 and on sixteen sampling plates during sampling period 3, but was not quantified. However, it was estimated that up to 25% of the surface area may have been subject to grazing on some plates. Some gastropods were seen on the plates when the plates were removed from both Lake Balannup and Lake Mt Brown.

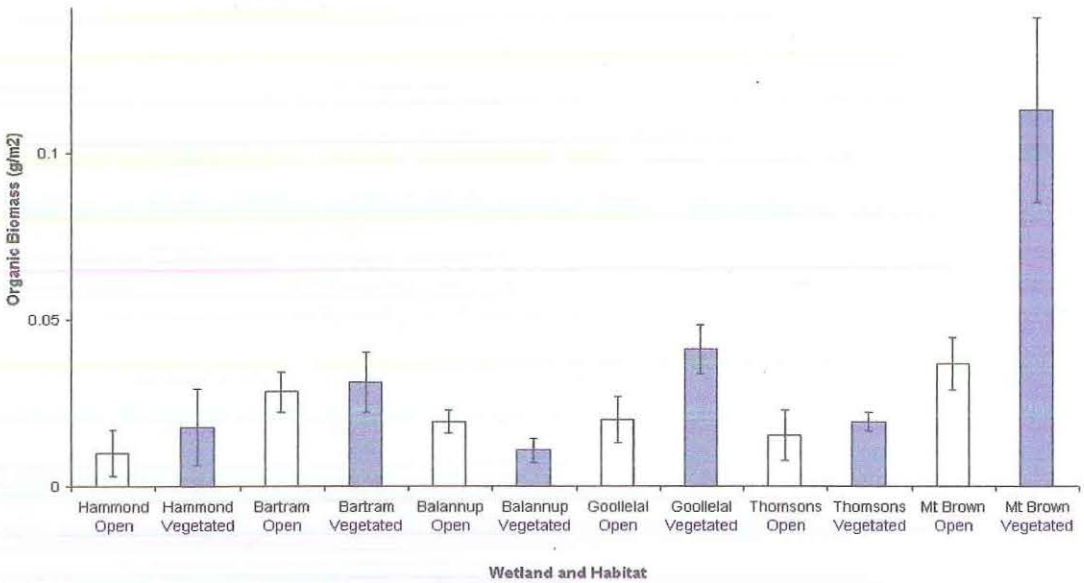


Figure 3.3 Mean Organic Biomass for all 6 wetlands in open and vegetated habitats. Significant differences existed both between wetlands (0.000, 5df, $p<0.05$) and between wetland habitats (0.031, 1df, $p<0.05$), with vegetated habitats having significantly higher organic biomass. Lake Mt. Brown had the highest organic biomass. (Bars indicate ±SE, n=12).

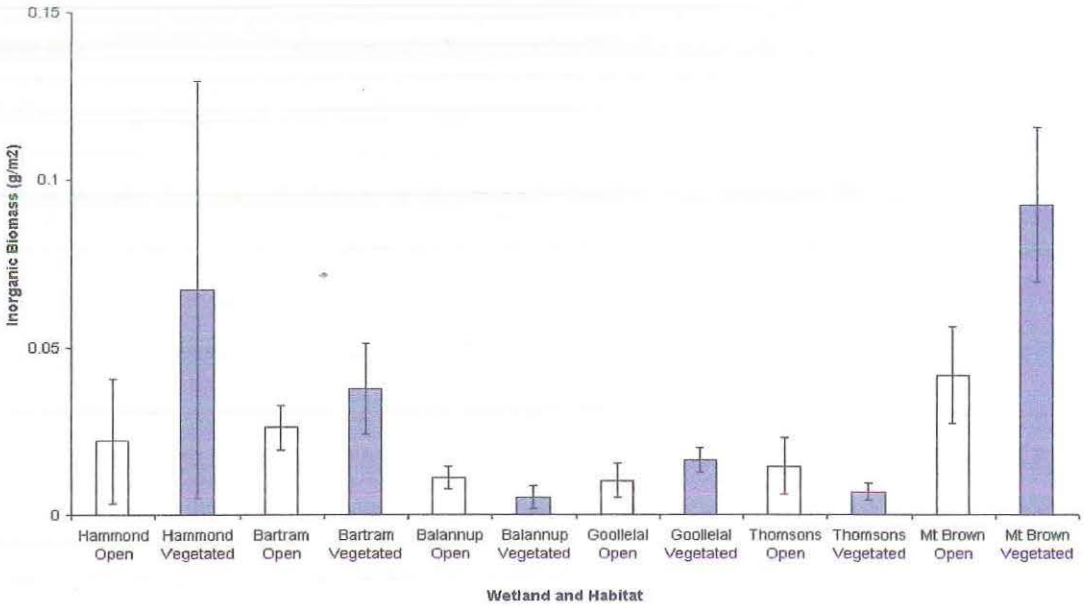


Figure 3.4 Mean Inorganic Biomass for all 6 wetlands in open and vegetated habitats. Significant differences in inorganic biomass were identified between wetlands (0.001, 5df, $p<0.05$), with Lake Mt Brown having the highest inorganic biomass. There was no significant difference of inorganic biomass between habitat types (0.421, 1df, $p>.05$). (Bars indicate ±SE, n=12).

3.2.2 Relative Percentage Cover

Significant differences in the biofilm mean relative percentage cover were identified between wetlands (Figure 3.5). Algae dominated both Lake Mount Brown and Lake Thomsons biofilms in both habitats, with values around 50%. The relative percentage algal cover was significantly different between wetlands (0.000, 5df, $p<0.05$), but not between habitat types (0.466, 1df, $p>0.005$), indicating that the proportion of algae within a wetland would not differ between habitat types. Post-hoc testing confirmed that Lake Mt. Brown was significantly different from all wetlands (5df, $p<0.05$) except for Lake Thomsons (0.988, 5df, $p>0.005$). Lake Thomsons was similar to Lake Goollelal and Lake Balannup (0.133, 5df, $p>0.05$; 0.096, 5df, $p>0.05$), but significantly different from both Hammond Road CWS (0.034, 5df, $p<0.05$) and Bartram Road CWS (0.025, 5df, $p<0.05$).

The relative fungal cover was significantly different between habitat types (0.015, 1df, $p<0.05$), but not between wetlands (0.064, 5df, $p>0.05$). Vegetated habitats, irrespective of the wetland, had more fungi cover than the open habitats. Fungi dominated in vegetated habitats of Hammond Road CWS, Bartram Road CWS, Lake Balannup and Lake Goollelal.

In contrast, the open habitats in Hammond Road CWS, Bartram Road CWS, Lake Balannup and Lake Goollelal were bacterially dominated. The open habitats of Bartram Road CWS and Lake Balannup had over 40% cover, and the open habitats of Hammond Road CWS and Lake Goollelal biofilms were bacterial dominated with over 50% cover. The relative cover of bacteria was significantly different between habitat types (0.001, 1df, $p<0.05$) and between wetlands (0.030, 5df, $p<0.05$). Post-hoc testing confirmed that there was a significant difference in relative bacterial cover between Lake Goollelal (41% cover) and Lake Mt. Brown (21% cover) (0.040, 5df, $p<0.05$), with no differences between all other wetlands (5df, $p>0.05$).

Despite comparable water quality between Lake Thomsons, Hammond Road CWS and Bartram Road CWS, the relative biofilm composition was very different. Both CWSs were fungal/bacterial dominated whereas Lake Thomsons was algal dominated in both habitat types.

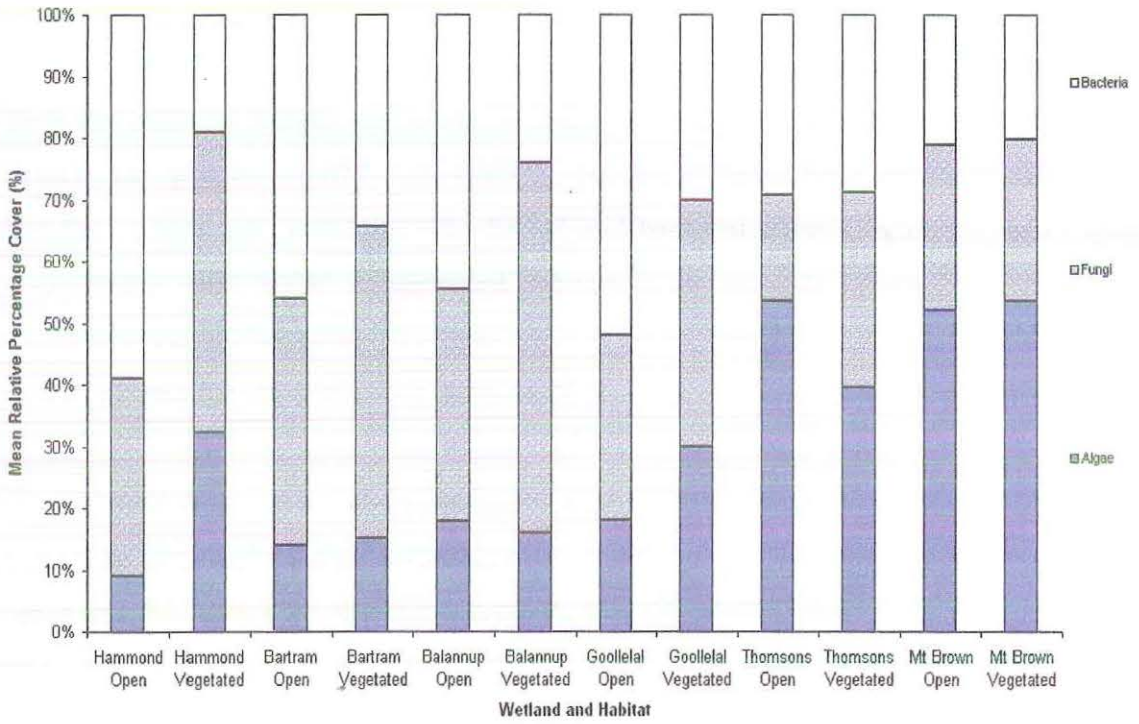


Figure 3.5 Mean Relative Percentage Cover of Algae, Fungi and Bacteria for all Wetlands Habitats. Algae dominate Lake Mt. Brown and Lake Thomsons biofilms. Open habitat biofilms of Hammond Road CWS, Bartram Road CWS, Lake Balannup and Lake Goollelal are bacterial dominated, while the vegetated habitats are fungal dominated. Standard errors were not shown for clarity. These errors can be seen in Figures 3.6 to 3.8.

3.2.3. Absolute Percentage Cover

As indicated by the relative percentage cover, Lake Mt. Brown and Lake Thomsons had higher absolute percentage cover of algae compared to the other wetlands (Figure 3.6). This difference between wetlands was significant (0.000, 5df, $p<0.05$), with post-hoc testing confirming that Lake Mt. Brown was significantly different from all wetlands (5df, $p<0.05$) except for Lake Thomsons (0.509, 5df, $p>0.05$), which was not significantly different from any other wetland (5df, $p>0.05$).

Both the absolute fungal and bacterial cover were significantly different between habitats (0.001, 1df, $p<0.05$; 0.027, 1df, $p<0.05$), but not different between wetlands (0.219, 5df, $p>0.05$; 0.059, 5df, $p>0.05$). The fungal and bacterial cover (Figure 3.7 and Figure 3.8 respectively) were higher in vegetated habitats in all wetlands except for Hammond Road CWS, in which the bacterial cover was lower in the vegetated habitat. All wetlands had similar overall percentage cover of either component.

The chlorophyll *a* results, an indication of algal biomass, showed patterns for most wetlands similar to the absolute algal percentage cover data. Vegetated habitats of Lake Mt Brown again had the highest chlorophyll *a* biomass, with Hammond Road CWS, Bartram Road CWS and Lake Balannup having similarly low chlorophyll 'a' biomass (Figure 3.9). Despite this similarity, the chlorophyll *a* biomass was significantly higher in the vegetated habitats (0.034, 1df, $p<0.05$), whereas the absolute percentage algal cover was significantly different between wetlands but not between habitat types. An additional anomaly in the comparison between algal percentage cover and the chlorophyll *a* biomass results occurred in Lake Thomsons, where the chlorophyll *a* results suggested a much lower algal biomass than indicated by the absolute percentage cover.

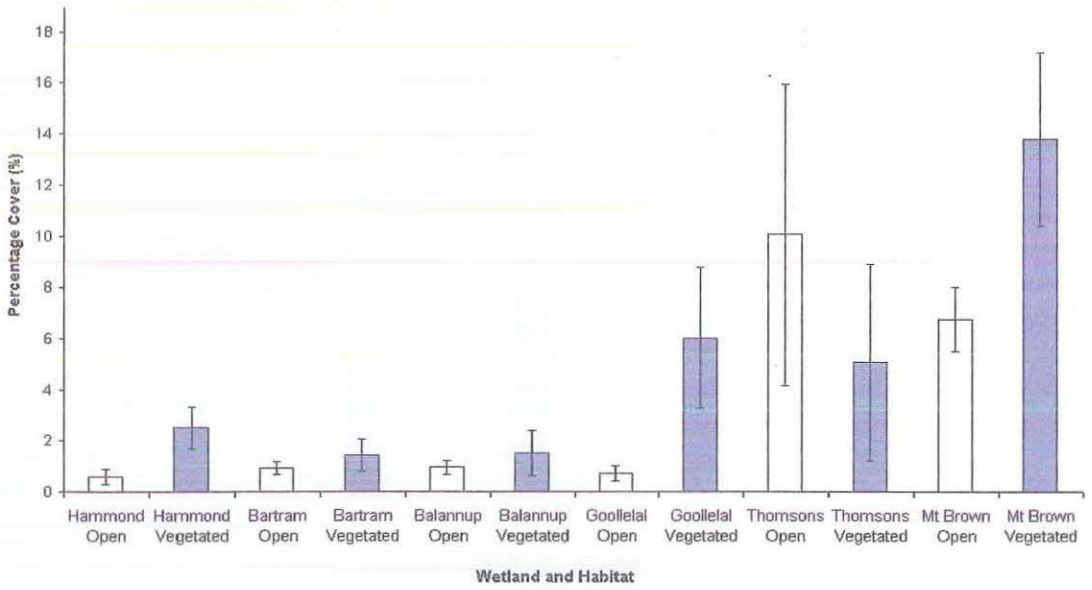


Figure 3.6 Algae Mean Percentage Cover (absolute). Results indicated significant differences between wetlands (0.000, 5df, $p<0.05$). Lake Mt Brown and Lake Thomsons had greater algal cover than the other wetlands. Note change in scale (Bars indicate ±SE, n=12).

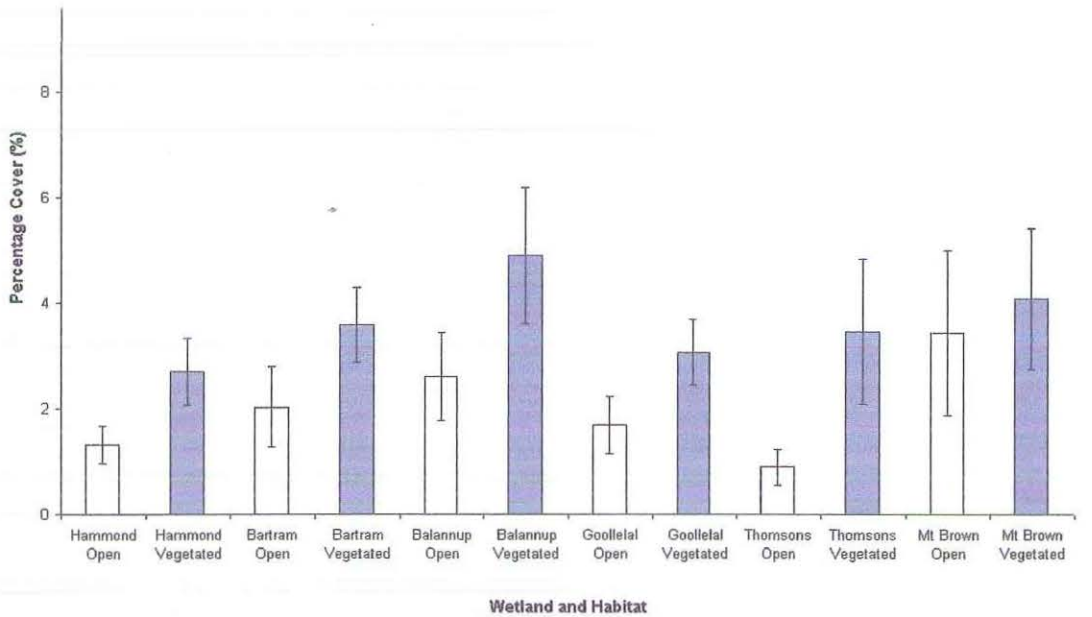


Figure 3.7 Fungi Mean Percentage Cover (absolute). Vegetated habitats had significantly higher fungal cover than open habitats in all wetlands (0.001, 1df, $p<0.05$). Note change in scale (Bars indicate ±SE, n=12).

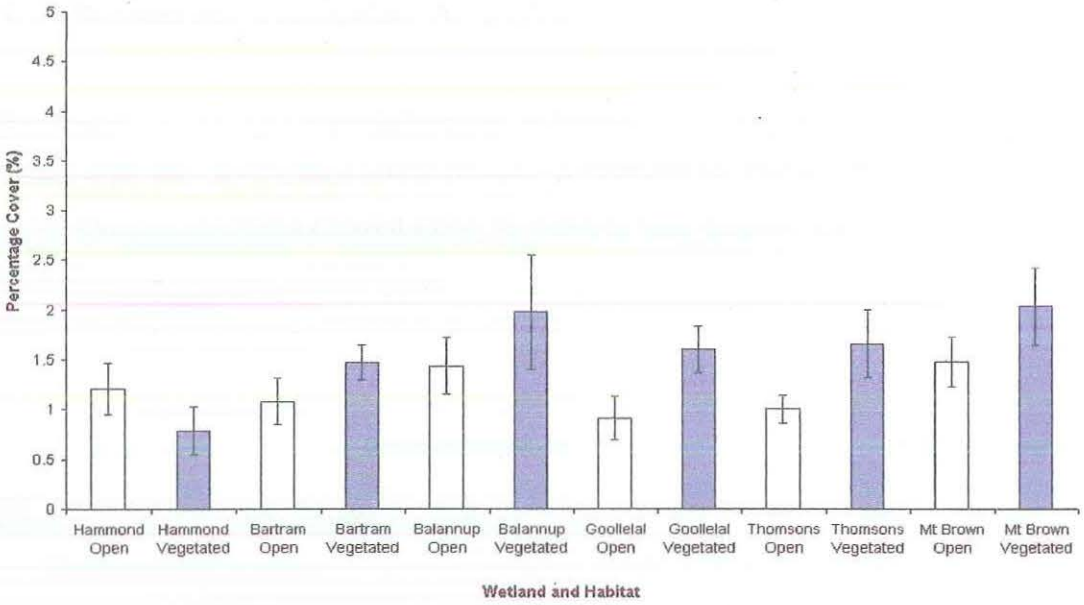


Figure 3.8 Bacteria Mean Percentage Cover (absolute). Vegetated habitats had significantly higher bacterial cover than open habitats overall (0.027, 1df, $p<0.05$), except for in Hammond Road CWS. Note change in scale (Bars indicate ±SE, n=12).

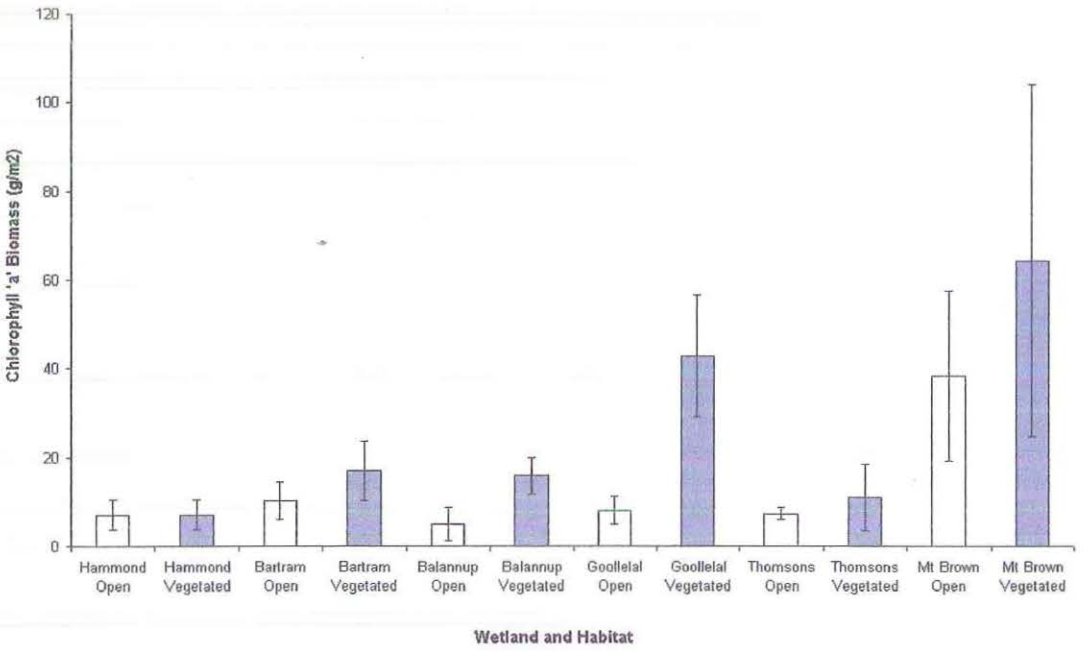


Figure 3.9 Mean Chlorophyll *a* Biomass. Vegetated habitats had significantly higher chlorophyll 'a' biomass than open habitats (0.034, 1df, $p<0.05$). (Bars indicate ±SE, n=4).

3.2.4 Biomass and Composition ANOVA Summaries

Summaries of the significant differences in biomass and composition from ANOVA are shown below with the significance levels (P-values) (Table 3.1). The results confirm high variability in biofilms on the Swan Coastal Plain, in terms of both biomass and composition, and between wetlands and wetland habitat types.

	Between Wetlands					Between Habitats				
Variable	**d.f.**	**Mean Square**	**F-Value**	**P-Value**		**d.f.**	**Mean Square**	**F-Value**	**P-Value**	
Percentage Organic Biomass (g/m^2)					NS					NS
Organic Biomass (g/m^2)	5	.113	11.201	**.000**	*	1	.04811	4.784	**.031**	*
Inorganic Biomass (g/m^2)	5	.06063	4.450	**.001**	*					NS
Algae % Composition (absolute)	5	.255	7.202	**.000**	*					NS
Fungi % Composition (absolute)					NS	1	.07653	11.706	**.001**	*
Bacteria % Composition (absolute)					NS	1	.006935	5.075	**.027**	*
Chlorophyll 'a' (g/m^2)					NS	1	.899	4.986	**.034**	*
Algae % Composition (relative)	5	.727	6.652	**.000**	*					NS
Fungi % Composition (relative)					NS	1	.566	.6130	**.015**	*
Bacteria % Composition (relative)	5	.200	2.600	**.030**	*	1	.901	11.702	**.001**	*

* = Statistically Significant NS = Not Significant

Table 3.1 2-Way ANOVA Significance for all factors with the P-values indicated. Wetland and Habitat interactions are not shown as no significant interaction terms occurred.

3.3 FRP UPTAKE KINETICS

Only two of the five batch-culture systems showed FRP changes that met the significance criterion. The 400μgL^{-1} system showed a net FRP uptake, while the 100μgL^{-1} system yielded a net FRP export (Figure 3.10). The changes in the remaining three systems failed to meet the uptake significance criterion.

FRP uptake at the highest concentration (400μgL^{-1}) was 14.56μghr^{-1}, equivalent to 1.67$\mu gmg^{-1}hr^{-1}$ when normalised to the mean tank biomass for the system (Table 3.2). FRP export recorded in the 100μgL^{-1} system was 6.66μghr^{-1}, equivalent to 0.20$\mu gmg^{-1}hr^{-1}$ when normalised to the mean tank biomass for the system.

Based on the FRP uptake results, it can be inferred that the maximum potential for a wetland to uptake FRP at 400μgL^{-1} is equal to or greater than 1.67$\mu gmg^{-1}hr^{-1}$. If this uptake rate is used to estimate the potential for a wetland to remove FRP, normalised to wetland biofilm biomass, Lake Mt. Brown vegetated habitat would have the highest potential FRP uptake rate at 189.2$\mu gm^{-2}hr^{-1}$ (Table 3.3). Lake Mt. Brown would also have the highest potential wetland uptake rate of all open habitats at 61.6$\mu gm^{-2}hr^{-1}$. The Lake Goollelal vegetated habitat would have the second highest potential FRP uptake rate at 68.8$\mu gm^{-2}hr^{-1}$, and Hammond Road CWS open habitat the lowest at 16.6$\mu gm^{-2}hr^{-1}$.

Additionally, because vegetated habitats were found to have higher organic biomass, FRP uptake rates normalised to biomass would be higher in vegetated habitats than in open habitats, with the exclusion of Lake Balannup (Figure 3.11). Vegetated habitats would have greater surface area because of the additional surface area for biofilm growth provided by macrophyte stems, thereby heightening the FRP uptake within a vegetated habitat for a given area.

The two further FRP uptake experiments indicated that similar FRP uptake rates were found under high PAR intensity (Appendix C), and that tanks not containing BBM were potentially nutrient limited (Appendix D).

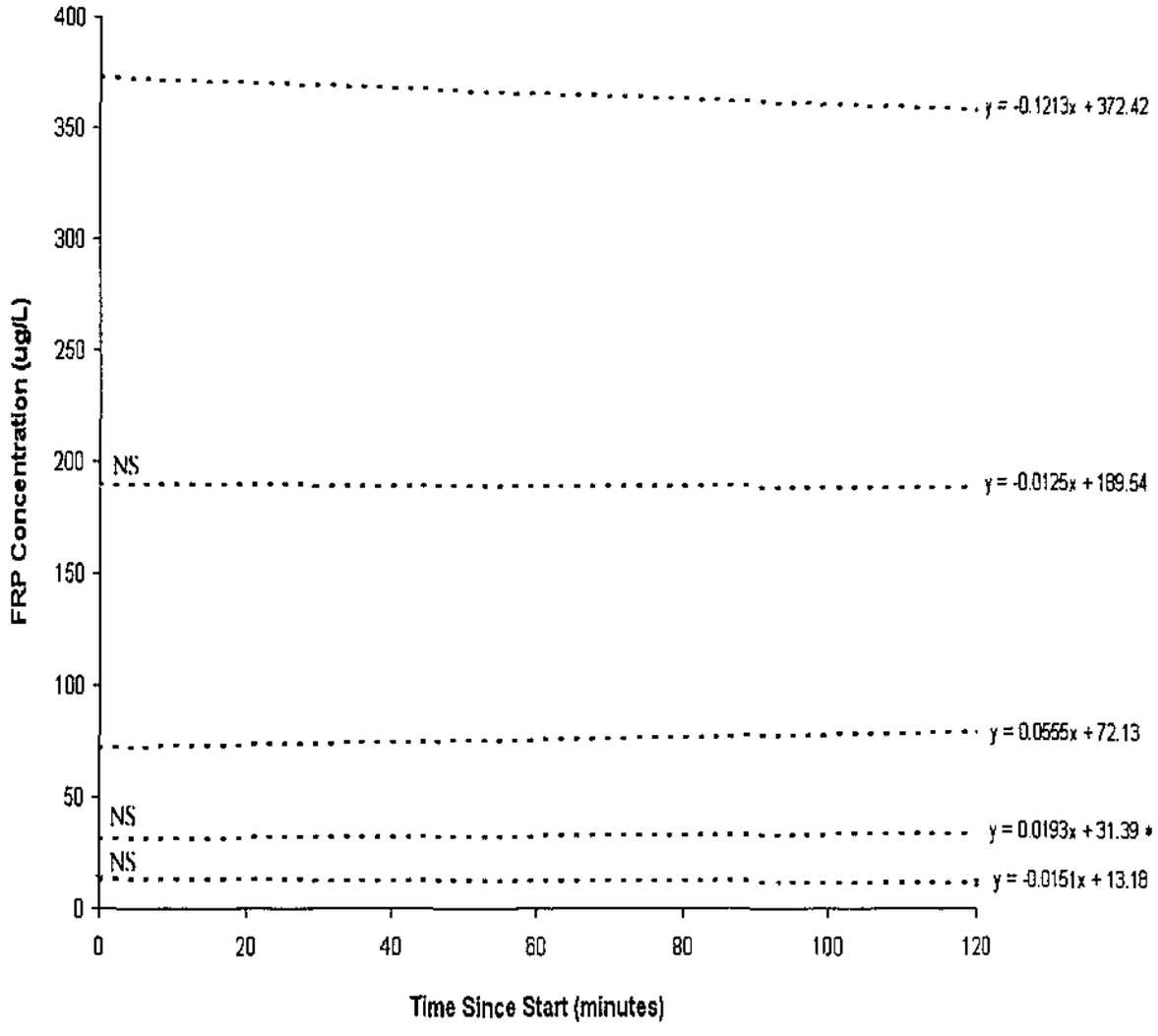


Figure 3.10 FRP Uptake Kinetics of Biofilm at 5 concentrations. The regression shown is the mean regression of all tanks in the given concentration. Two concentrations recorded uptakes that met the significance criteria. The remaining three concentrations did not meet the criteria (marked by NS). The 400μgL^{-1} concentration had an FRP uptake, while the 100μgL^{-1} concentration had a net FRP export. (n=5, *n=4).

FRP Concentration	Mean Tank Uptake Rate ($\mu g hr^{-1}$)	Mean Organic Biomass (mg)	FRP Uptake Rate per mg Organic Biomass per hour. ($\mu g mg^{-1} hr^{-1}$)
$400 \mu g L^{-1}$	14.56	8.7	**1.67**
$200 \mu g l^{-1}$	NS	17.6	NS
$100 \mu g L^{-1}$	-6.66	33.8	**-0.20**
$50 \mu g L^{-1}$	NS	7.8	NS
$<50 \mu g L^{-1}$	NS	12.5	NS

Table 3.2 Mean FRP Uptake normalised to Concentration Biomass. The highest concentration had the highest FRP uptake rate normalised to biomass.

Wetland and Habitat		Mean Organic Biomass ($mg m^{-2}$)	Wetland Habitat Uptake Rate ($\mu g m^{-2} hr^{-1}$)
Hammond Road CWS	Open	10.1	**16.6**
	Vegetated	17.9	**29.9**
Bartram Road CWS	Open	28.4	**47.5**
	Vegetated	31.3	**52.4**
Lake Balannup	Open	19.4	**32.5**
	Vegetated	10.8	**18.1**
Lake Goollelal	Open	20.1	**33.6**
	Vegetated	41.1	**68.8**
Lake Thomsons	Open	15.3	**25.6**
	Vegetated	19.4	**32.5**
Lake Mt. Brown	Open	36.8	**61.6**
	Vegetated	113.1	**189.2**

Table 3.3 Potential Maximum Uptake Rates of FRP for all wetland habitats. The potential uptake rates were calculated using the highest uptake rate from Figure 3.2. Lake Mt. Brown had the highest FRP uptake rates of both open and vegetated habitats because of high organic biomass.

CHAPTER 4: DISCUSSION

4.1 BIOFILM BIOMASS

Significant differences in biofilm organic and inorganic biomass existed between wetlands, with the biofilm organic biomass also significantly different between habitat types. The organic and inorganic biomass of Bartram Road CWS fell within the ranges observed for the natural wetlands. The organic biomass of Hammond Road CWS was lower than the ranges between the natural wetlands. The results for Bartram Road CWS were similar to results recorded by Duncan and Groffman (1994), who found that microbial biomass from soil cores taken from stormwater CWSs and natural wetlands fell within the ranges observed in natural wetlands. High variability of both organic and inorganic biofilm biomass between natural wetlands indicate that a comparison of CWS biofilm biomass to a single natural wetland would not be appropriate. Higher organic biomass in Bartram Road CWS compared to Hammond Road CWS may indicate that biofilm biomass increases with the age of the system , as also indicated by CWS conceptual models of phosphorus removal.

Significantly higher biofilm biomass in the vegetated habitats indicates that maximising the area of vegetated habitat would maximise biofilm biomass, and therefore increase FRP removal. Furthermore, the macrophyte stems of vegetated habitats provide additional surface area for biofilm growth in a given area. However, maximising the area of vegetated habitat should be balanced with the need for open habitats, and the functions and benefits that these habitats perform, such as aesthetics. The only exception to this trend of higher organic biomass in the vegetated habitat was in Lake Balannup, in which the open habitat had higher organic biofilm biomass. One likely reason for this could be attributed to the *Lemna spp.*

covering the open water habitat, resulting in the open water habitat having characteristics of vegetated habitats.

Despite comparability of biofilm biomass between CWSs and natural wetlands, biofilm biomass in all the Swan Coastal Plain wetlands sampled was low. Cronk and Mitsch (1994) calculated organic biomass at between 1-5gm^{-2} over a 2-week period on glass slides in a stormwater CWS in Illinois. Robertson *et al.* (1997) calculated biofilm dry biomass at 3-60$gm^{-2}wk^{-1}$ from untreated pine blocks placed 20cm above the sediment in billabongs on the Murrumbidgee River, New South Wales. In wetlands on the Swan Coastal Plain the organic biomass was significantly lower at 0.01-0.11gm^{-2} for a 2-week period. The values obtained by Cronk and Mitsch (1994) and Robertson *et al.* (1997) are significantly higher, suggesting that the biofilm biomass in both stormwater CWSs and natural wetlands on the Swan Coastal Plain are low. There is no clear indication as to why biofilm biomass is lower in stormwater CWSs and natural wetlands on the Swan Coastal Plain.

Similarly, Baskaran *et al.* (1993) calculated the standing crop of biofilm at 0.5-4.6gm^{-2} in a Melbourne wastewater CWS. Although wastewater CWS biofilms would likely be significantly different compositionally because of the different nature of the influent, these data indicate that the biofilm biomass in stormwater CWSs on the Swan Coastal Plain is also lower than wastewater CWSs in eastern Australia.

Conceptual models of phosphorus removal for Australian stormwater CWSs (e.g. White and Wiese, 1997, cited in JDA Consultant Hydrologists, 1997; DLWC, 1998; Lantzke *et al.*, 1999) were developed in the eastern states of Australia, where the systems have higher biofilm biomass. These results suggest that biofilm in phosphorus removal in stormwater CWSs on the Swan Coastal Plain may be less significant than the conceptual models anticipate because of lower biomass.

The organic proportion of biofilm biomass was not significantly different between wetlands or between habitat types, ranging between 40-60%. This indicates that the inorganic and organic components have an equally important part in the biofilm structure. Rough estimates of

organic biomass could therefore be calculated based on the biofilm dry weight alone. In contrast, Sheldon and Walker (1997) found biofilm organic percentage biomass ranging from 16-26% in Cooper Creek (South Australia) and at 18% in the lower River Murray (South Australia) using natural substrates. The organic proportion of biofilms in wetlands on the Swan Coastal Plain may therefore be higher than in the southeastern states of Australia. However, it should be noted that the differences in the organic proportion may be related to the substrate type.

Lake Mt Brown was significantly higher in organic and inorganic biofilm biomass than most other wetlands. High salinity was the most distinguishing feature of Lake Mt Brown which separated it from the other wetlands. Higher organic and inorganic biomass in Lake Mt Brown should not suggest, however, that CWS design should aim to produce saline stormwater systems. Increasing biofilm biomass would be better achieved by other mechanisms such as the increasing the surface area for biofilm growth.

4.2 BIOFILM COMPOSITION

Composition characteristics appeared principally a reflection of the PAR intensity at the sediment layer, with biofilm composition moving from algal dominated to bacterial/fungal dominated with decreasing PAR intensity. Dissolved humic substances (gilvin) originating from the breakdown of plant material cause brown staining of wetland waters on the Swan Coastal Plain (Davis *et al.*, 1993). This staining is often referred to as colour, with increased loads of humic substances resulting in increased colour. Algae dominated the biofilms of Lake Thomsons and Lake Mt. Brown, which were observed to have comparatively low colour and shallow water, resulting in higher PAR intensity at the sediment. Bacteria and fungi dominated the biofilms of Hammond Road CWS, Bartram Road CWS, and Lake Balannup, which were all observed to have high colour and increased depth resulting in reduced PAR intensity at the

sediment. Within the wetlands observed to be highly coloured, bacteria dominated the open habitats and fungi dominated the vegetated habitats.

The colour of Lake Goollelal was observed to be between the highly and lightly coloured wetlands. The vegetated habitat of Lake Goollelal recorded higher algal cover in the vegetated habitat. In contrast, the open habitat composition of Lake Goollelal had similar bacterial domination to the open habitats of the highly coloured wetlands. The increased algal cover in the vegetated habitat was likely a result of reduced depth in the vegetated habitat, thereby increasing PAR at the sediment layer. Hammond Road CWS also indicated similar heightened algal composition in the vegetated habitats.

That PAR intensity as a major factor determining biofilm composition is further supported by the relative biofilm compositions of Lake Thomsons, Hammond Road CWS and Bartram Road CWS. Hammond Road CWS and Bartram Road CWS receive influent stormwater from a drain leading into Lake Thomsons, and thus are comparable in terms of most physico-chemical properties. However, the relative composition was significantly different. Lake Thomsons, a shallow and lightly coloured wetland, had biofilms that were algal dominated. In contrast, both CWSs were highly coloured and both had biofilms that were fungal/bacterial dominated. This suggests that differences in biofilm composition may not be primarily related to physico-chemical attributes. This inferred relationship between high colour and low algal dominance, or alternatively low colour and high algal dominance, suggests that PAR intensity at the sediment layer may be the primary factor determining biofilm composition.

These results are supported by previous biofilm studies that state high PAR intensity tend to result in algal dominated biofilms (Lock *et al.*, 1984), and that biofilms become bacterial dominated at low PAR intensities (Blenkinsopp and Lock, 1994). Mosisch *et al.* (1999) also reported similar increased algal composition (measured by chlorophyll *a*) with increasing PAR intensity. It appears that similar patterns of biofilm composition occurred in the wetlands studied, with water colour and depth affecting the PAR intensity at the sediment layer.

Lawrence and Breen (1998) state that dissolved nutrients (such as FRP) are primarily taken up by epiphytic and benthic algae. If true, then engineering of CWS PAR intensity through the application of this 'PAR-Composition pattern' may allow the promotion of an algal dominated biofilm composition, thus optimising FRP removal.

The mean biofilm cover (absolute) did not exceed 25% for any wetland habitat, with cover generally at 10-12%. Silyn-Roberts and Lewis (1997) calculated similar results with biofilm coverage on glass substrates not exceeding 22% after 6 weeks in the field in a wastewater CWS. Similar cover with differences in the sampling time indicate that the biofilm colonisation rate may be higher in stormwater CWSs on the Swan Coastal Plain. However, these differences may simply be due to CWS design and influent characteristics.

The results for chlorophyll *a* did not exceed $16\mu gm^{-2}$, with similar patterns to algal cover. Chlorophyll *a* recorded by Sheldon and Walker (1997) ranged between 0.5-$9mgm^{-2}$ in Cooper Creek and around $100mgm^{-2}$ in the River Murray in eastern Australia, with the higher chlorophyll *a* attributable to higher total biofilm biomass. The algal percentage cover was significantly different between wetlands, but not between habitat types, whereas chlorophyll *a* was significantly different between habitat types, but not between wetlands. These discrepancies raise an interesting debate on the use of chlorophyll *a* as the standard measure for estimating standing and fixed algal biomass. Direct counting is the most accurate measure because it does not discriminate between algal species, whereas analysis of chlorophyll *a* may indicate higher algal biomass for species that have higher chlorophyll *a* content within their cells. The clearest discrepancy of this kind can be noted in Lake Thomsons, where analysis of chlorophyll a biomass indicated algal biomass significantly lower than most wetlands, whereas direct counting methods indicated that the algal content is the second highest of all wetlands sampled.

4.2.1 Limitations on Biofilm Composition Results

The comparison of lightly coloured versus darkly coloured wetlands were based on comparative observations made of the water clarity during the research period. The PAR intensity at the sediment layer was not measured, and this therefore represents a limitation to interpreting the composition results relating to the 'PAR-Composition pattern'. The compositional analysis of biofilms for all sampling periods from all wetlands were conducted after most of the wetlands had become dry, and therefore measuring PAR intensities were not feasible when it was realised that this may have been the determining factor.

4.3 BIOFILM FRP UPTAKE KINETICS

The maximum potential FRP uptake by biofilm was equal to or greater than 1.67$\mu gmg^{-1}hr^{-1}$. The maximum potential FRP uptake normalised to the mean biomass of each wetland habitat resulted in potential wetland FRP removal rates ranging between 16.6-189.2$\mu gm^{-2}hr^{-1}$. The highest potential uptake rate, recorded for Lake Mt Brown, was uncharacteristically higher than for the other wetlands, a result of the high biofilm biomass resulting from the wetland's physico-chemical differences previously outlined. The second highest uptake rate was significantly lower at 61.6$\mu gm^{2-1}hr^{-1}$. Extrapolation of the potential wetland FRP removal rates results in potential uptake rates ranging from 2.8-31.8$mgm^{-2}wk^{-1}$.

Mitsch *et al.* (1995) estimated the combined water column and biofilm FRP uptake at 4-6$mgm^{-2}wk^{-1}$ from a stormwater CWS on freshwater riparian marshes in Illinois (United States). Cronk and Mitsch (1994) estimated biofilm FRP uptake slightly lower at 1-3$mgm^{-2}wk^{-1}$ for the same system. FRP uptake rates from both Mitsch *et al.* (1995) and Cronk and Mitsch (1994) are similar to the lowest positive uptake rates found in this study, but considerably lower than for the higher uptake rates. The results indicate that despite having lower biofilm organic biomass, the potential biofilm FRP uptake capacity in both CWSs and natural wetlands on the

Swan Coastal Plain are higher. However, in relation to CWS conceptual model mechanisms, Mitsch *et al.* (1995) concluded that most influent phosphorus was retained through sedimentation and by macrophytes, with a lesser amount removed by biofilms. In contrast to this, higher biofilm FRP uptake and the diminished role of sedimentation (see p.7) for CWSs on the Swan Coastal Plain indicate that long-term phosphorus removal by biofilm may be highly significant.

Despite the emphasis given to the maximum potential FRP uptake rate, it also must be considered that FRP uptake by biofilm appears to be negligible at low concentrations. Three of the batch-culture systems indicated no FRP removal. It must therefore be concluded that FRP uptake at low concentrations may be negligible, given that the rate of uptake is concentration dependent (Kadlec, 1997). The biofilm FRP export recorded by one of the batch-culture systems would not occur over long time periods within a wetland due to the limited quantity of phosphorus bound within the biofilms. The export recorded was possibly a result of diffusion of FRP from the biofilm when placed in a lower FRP concentration.

4.3.1 Limitations of FRP Uptake Results

Conversion of the highest normalised uptake rate to the mean wetland biofilm biomass of each habitat type enabled estimates of the potential maximum biofilm FRP removal capacity of each system. However, this conversion assumes biofilm compositional uniformity between wetlands and between habitat types. The results should be used with more caution for Lake Mt Brown and Lake Thomsons, which have biofilm composition dissimilar to the open water zones of the Hammond Road CWS from which the biofilm for FRP uptake experiments was obtained. Alternatively, if algae were the dominant removal mechanism within the biofilm, FRP removal would likely be higher for these wetlands than the results indicate.

The biofilms used for the FRP uptake experiments may have been phosphorus-saturated within the wetland prior to the commencement of the FRP uptake experiments. If this were true, the

potential FRP uptake rates may be higher for all wetlands than the results indicate. It was for this reason that the maximum potential FRP uptake rates for each wetland are stated as being equal to or greater than the values obtained.

4.4 FAILURE OF STORMWATER CWSs ON THE SWAN COASTAL PLAIN

The FRP uptake potential of biofilm was comparable or higher than previous published literature has suggested; indicating that high FRP uptake within Swan Coastal Plain stormwater CWSs was possible. Additionally, because of the diminished role of sedimentation, the role of phosphorus removal by biofilm is heightened.

The biofilm biomass of the CWSs were generally comparable to the natural wetlands in terms of biofilm biomass, but with increased biomass possible. The biofilm organic biomass was higher in the older CWS, indicating that biofilm development may take longer than previously anticipated. Additionally, CWSs on the Swan Coastal Plain have not aimed to optimise the surface area for biofilm development. The addition of surface area for biofilm development will likely increase FRP removal.

Despite similar biomass to the natural wetlands sampled, the composition of both CWSs was similar only to the highly coloured natural wetlands. It is possible that poor FRP removal performance in the CWSs were reflective of a biofilm composition that was poor in FRP removal. Increasing the PAR intensity, either by reducing depth or by removing the water colour, may increase FRP removal from these systems by shifting the biofilm to an algal dominated composition. Future research on CWS biofilms on the Swan Coastal Plain should aim to further investigate the effect of increasing PAR intensity on biofilm composition and FRP removal.

4.5 MAXIMISING BIOFILM BY CWS DESIGN AND MANAGEMENT

Maximising the surface area for biofilm growth and increasing PAR intensity have been identified as the two major controllable factors that may increase FRP removal by biofilm in stormwater CWSs. The manipulation of both of these factors should be done in tandem for optimal FRP removal.

Maximising biofilm biomass would be best achieved by maximising the area of vegetated habitat, given that vegetated habitats have significantly higher biofilm biomass. However, this management option should be balanced with recognising the nutrient removal functions and associated secondary benefits that open habitats perform. Additionally, the density of the vegetation should be monitored in order not to increase shading above densities at which the algal proportion would be reduced. Wastewater CWSs increase the surface area for biofilm growth by the addition of the substratum such polyethylene or plastic spheres (Jones, 1995; Rusten *et al.*, 1998; Pastorelli *et al.*, 1999), PVC plates (Baskaran *et al.*,1993), active carbon particles (Beyenal and Tanyolac, 1996) or by using a gravel substrata (Mann and Bavor, 1993; Polprasert *et al.*, 1998). Similar surface area maximising processes could be adopted by stormwater CWSs on the Swan Coastal Plain in order to increase FRP removal. This process may or may not be suitable for stormwater CWSs, given that such options may not be compatible with associated secondary benefits such as recreation and habitat creation. However, the biofilm biomass in Swan Coastal Plain wetlands appears low and options such as this may be considered necessary. Access restrictions to both humans and wildlife would be required to counter such compatibility problems. A combination of the addition of substrata and an increased area of vegetated habitat will likely increase FRP removal.

Increasing PAR intensity to the sediment layer could be achieved either by the removal of colour and turbidity, the manipulation of wetland depth, or a combination of the two. Removal of colour would likely require chemical treatment to precipitate the humic substances or the iron that cause the colour. Decreasing wetland depth would likely be the most convenient mechanism for increasing PAR intensity at the sediment. Decreasing wetland depth would

likely increase the algal dominance in biofilms, potentially increasing FRP removal. Minimising wetland depth would also have the advantage of increasing the surface area to volume ratio, thereby increasing the biofilm biomass relative to the water volume. Of the CWS literature reviewed (both stormwater and wastewater), wetland depths ranged from 26cm (Pollard *et al.*, 1995) to 1.7m (Rusten *et al.*, 1998), with most systems having depths at 0.5m. Both Hammond Road CWS and Bartram Road CWS have a maximum depth at 1-1.5m, as well as high colour. Decreasing wetland depth would reduce hydraulic residence time, which is the time which water is held within a system. Manipulating wetland depth would need to be balanced with the need for maintaining high HRT, or alternatively the hydraulic residence time could be maintained by increasing the area of the system.

4.6 CONCLUSIONS

The specific aims of this research project were to:

- Compare the biofilm biomass of stormwater CWSs with natural wetlands of the Swan Coastal Plain.
- Compare the biofilm composition of stormwater CWSs with natural wetlands, and
- Determine the FRP uptake potential of biofilm, in order to determine the potential contribution by biofilm to FRP removal.

Both biofilm biomass and biofilm composition were found to be highly variable between wetlands and between habitat types in the natural wetlands sampled on the Swan Coastal Plain. Both biofilm biomass and biofilm composition of the stormwater CWSs sampled generally fell within the ranges observed within the natural wetlands, with the exception of the organic biomass of the open habitat of Hammond Road CWS, which fell below the natural wetland ranges. Significantly higher biofilm biomass in the vegetated habitats indicated that the maximising of biofilm biomass would be best achieved by maximising the area of vegetated

habitat. However, this should be balanced with the need for open habitats and the phosphorus removal functions that these habitats perform.

Biofilm composition appeared to be a product of PAR intensity at the sediment layer, with water colour and water depth reducing PAR intensity. The biofilm composition of both stormwater CWSs were fungal/bacterial dominated in both habitat types, similarly to the highly coloured natural wetlands sampled. In contrast, the natural wetlands observed having low colour, combined with low water depth, had biofilms that were algal dominated. Low phosphorus removal efficiency of the CWSs may result from a biofilm composition that is poor in FRP removal. Engineering of algal dominated biofilms by the manipulation of CWS design to increase the PAR intensity may increase FRP removal.

The FRP uptake rate appeared to be concentration limited, with low FRP systems failing to indicate significant FRP removal during laboratory testing. In contrast, at high FRP concentrations (~400μgL^{-1}), significant FRP removal occurred. The FRP uptake potential of biofilm normalised to biofilm organic biomass was found to be comparable or higher than previous published literature had suggested, despite significantly lower biofilm organic biomass in both natural wetlands and stormwater CWSs.

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APPENDIX A

Unprocessed Biomass and Composition Data for All Wetlands

APPENDIX A

Unprocessed data for all wetlands in all sampling periods.

A (.) indicates that no sample was retrieved. Note that Lake Balannup was not sampled in Sampling Period 1 due to accessibility, and both Lake Thomsons and Lake Balannup were not sampled in Sampling Period 4 as both wetlands were dry.

Wetland	Habitat	Sampling Period	Algae % Cover	Fungi % Cover	Bacteria % Cover	Chlorophyll 'a' Biomass (g/m2)	Organic Biomass (g/m2)	Inorganic Biomass (g/m2)	Percentage of Organic Biomass Composition
Hammond CWS	Open	1	0.7	0.0	1.7	16.5	0.0042	0.0045	48.57
Hammond CWS	Open	1	0.0	0.0	1.9	.	0.0063	0.0017	78.13
Hammond CWS	Open	1	.	.	.	.	0.0855	0.2260	27.45
Hammond CWS	Open	2	0.0	0.0	0.6	2.8	0.0040	0.0007	84.21
Hammond CWS	Open	2	1.0	1.7	0.3	.	0.0045	0.0013	78.26
Hammond CWS	Open	2	0.0	3.7	0.7	.	0.0047	0.0038	55.88
Hammond CWS	Open	3	3.0	2.7	1.2	6.4	0.0013	0.0060	65.22
Hammond CWS	Open	3	0.0	1.0	1.9	.	0.0005	0.0047	9.52
Hammond CWS	Open	3	0.0	1.0	0.5	.	0.0035	0.0070	33.33
Hammond CWS	Open	4	1.7	1.7	0.7	2.5	0.0038	0.0058	39.47
Hammond CWS	Open	4	0.0	2.0	3.1	.	0.0030	0.0018	63.16
Hammond CWS	Open	4	0.0	0.7	0.7	.	0.0000	0.0010	0.00
Hammond CWS	Vegetated	1	8.7	0.0	0.8	5.3	0.0053	0.0062	45.65
Hammond CWS	Vegetated	1	1.3	1.7	0.5	.	0.0033	0.0000	100.00
Hammond CWS	Vegetated	1	.	.	.	.	0.0060	0.0000	100.00
Hammond CWS	Vegetated	2	1.0	5.0	1.0	3.6	0.0128	0.0060	68.00
Hammond CWS	Vegetated	2	3.0	2.0	0.2	.	0.0068	0.0023	75.00
Hammond CWS	Vegetated	2	3.0	7.0	0.7	.	0.0042	0.0002	94.44
Hammond CWS	Vegetated	3	2.7	2.3	2.8	16.9	0.0055	0.0098	36.07
Hammond CWS	Vegetated	3	0.0	1.7	0.6	.	0.0030	0.0047	38.71
Hammond CWS	Vegetated	3	.	.	.	.	0.0002	0.0052	4.55
Hammond CWS	Vegetated	4	1.3	1.0	0.6	2.3	0.0170	0.0170	50.00
Hammond CWS	Vegetated	4	0.0	3.3	0.6	.	0.1448	0.7508	16.16
Hammond CWS	Vegetated	4	4.0	3.0	0.1	.	0.0055	0.0045	55.00
Bartram CWS	Open	1	0.0	0.3	0.5	2.1	0.0068	0.0015	81.82
Bartram CWS	Open	1	.	.	.	.	0.0050	0.0015	76.92
Bartram CWS	Open	1	.	.	.	.	0.0057	0.0008	88.46
Bartram CWS	Open	2	0.0	0.7	0.1	4.4	0.0132	0.0090	59.55
Bartram CWS	Open	2	1.0	1.3	1.0	.	0.0488	0.0625	43.82
Bartram CWS	Open	2	0.3	1.0	0.6	.	0.0125	0.0085	59.52
Bartram CWS	Open	3	2.0	3.7	0.7	20.3	0.0148	0.0140	51.30
Bartram CWS	Open	3	2.0	8.3	2.6	.	0.0523	0.0440	54.29
Bartram CWS	Open	3	1.3	1.3	1.4	.	0.0397	0.0363	52.30
Bartram CWS	Open	4	1.7	1.7	1.9	13.9	0.0510	0.0430	54.26
Bartram CWS	Open	4	0.3	1.3	1.2	.	0.0608	0.0623	49.39
Bartram CWS	Open	4	0.7	0.7	0.8	.	0.0298	0.0295	50.21
Bartram CWS	Vegetated	1	3.0	4.0	1.6	16.2	0.0127	0.0060	68.00

Bartram CWS	Vegetated	1	0.0	0.0	0.9	.	0.0010	0.0011	48.81
Bartram CWS	Vegetated	1	.	.	.	.	0.0115	0.0015	88.46
Bartram CWS	Vegetated	2	1.7	3.3	1.5	3.2	0.0470	0.0375	55.62
Bartram CWS	Vegetated	2	0.3	2.7	0.8	.	0.0280	0.0218	56.28
Bartram CWS	Vegetated	2	0.0	5.3	1.5	.	0.0253	0.0185	57.71
Bartram CWS	Vegetated	3	1.7	5.0	1.9	35.5	0.0089	0.1376	6.08
Bartram CWS	Vegetated	3	0.3	1.0	0.7	.	0.0133	0.0142	48.18
Bartram CWS	Vegetated	3	0.7	2.0	1.6	.	0.0890	0.1023	46.54
Bartram CWS	Vegetated	4	6.3	7.3	1.6	12.8	0.0857	0.0598	58.93
Bartram CWS	Vegetated	4	0.3	5.3	2.6	.	0.0223	0.0120	64.96
Bartram CWS	Vegetated	4	.	.	.	.	.	.	.
L. Goollelal	Open	1	0.3	0.7	0.2	13.1	0.0038	0.0000	100.00
L. Goollelal	Open	1	0.7	3.7	1.4	.	0.0065	0.0015	81.25
L. Goollelal	Open	1	.	.	.	.	0.0130	0.0000	100.00
L. Goollelal	Open	2	2.0	1.3	2.0	3.9	0.0730	0.0185	79.78
L. Goollelal	Open	2	0.0	0.0	0.0	.	0.0192	0.0080	70.64
L. Goollelal	Open	2	0.3	3.0	1.3	.	0.0060	0.0028	68.57
L. Goollelal	Open	3	0.0	0.3	0.3	13.7	0.0045	0.0042	51.43
L. Goollelal	Open	3	0.0	0.0	0.2	.	0.0140	0.0042	76.71
L. Goollelal	Open	3	0.3	1.3	1.2	.	0.0183	0.0063	74.49
L. Goollelal	Open	4	1.7	0.0	0.2	1.6	0.0172	0.0108	61.61
L. Goollelal	Open	4	2.7	5.3	1.6	.	0.0655	0.0635	50.78
L. Goollelal	Open	4	0.0	3.0	1.6	.	0.0000	0.0023	0.00
L. Goollelal	Vegetated	1	6.0	4.0	0.6	22.2	0.0333	0.0042	88.67
L. Goollelal	Vegetated	1	1.3	3.7	1.8	.	0.0540	0.0087	86.06
L. Goollelal	Vegetated	1	.	.	.	.	0.0263	0.0022	92.11
L. Goollelal	Vegetated	2	1.0	4.7	1.3	.	0.0980	0.0257	79.19
L. Goollelal	Vegetated	2	3.0	2.0	1.5	.	0.0020	0.0045	30.77
L. Goollelal	Vegetated	2	0.0	5.3	2.0	.	0.0438	0.0148	74.79
L. Goollelal	Vegetated	3	0.7	2.0	1.5	68.9	0.0455	0.0240	65.47
L. Goollelal	Vegetated	3	3.7	1.0	1.5	.	0.0277	0.0137	66.87
L. Goollelal	Vegetated	3	2.7	1.7	1.6	.	0.0550	0.0157	77.74
L. Goollelal	Vegetated	4	0.0	0.7	0.3	37.3	0.0095	0.0070	57.58
L. Goollelal	Vegetated	4	27.3	7.3	2.3	.	0.0587	0.0415	58.60
L. Goollelal	Vegetated	4	20.3	1.3	3.2	.	0.0398	0.0345	53.54
L. Thomsons	Open	1	0.0	2.3	0.8	4.4	0.0110	0.0030	78.57
L. Thomsons	Open	1	.	.	.	.	0.0038	0.0005	88.24
L. Thomsons	Open	1	.	.	.	.	.	.	.
L. Thomsons	Open	2	3.7	0.0	0.9	8.5	0.0068	0.0085	44.26
L. Thomsons	Open	2	1.3	1.0	1.0	.	0.0130	0.0153	46.02
L. Thomsons	Open	2	7.3	0.3	1.4	.	0.0058	0.0050	53.49
L. Thomsons	Open	3	1.3	0.3	0.4	8.9	0.0515	0.0555	48.13
L. Thomsons	Open	3	12.7	0.3	1.5	.	.	.	.
L. Thomsons	Open	3	44.0	2.0	1.0	.	.	.	.
L. Thomsons	Open	4	.	.	.	.	.	.	.
L. Thomsons	Open	4	.	.	.	.	.	.	.
L. Thomsons	Open	4	.	.	.	.	.	.	.
L. Thomsons	Vegetated	1	2.3	7.3	1.6	3.6	0.0115	0.0010	92.00
L. Thomsons	Vegetated	1	0.0	4.0	2.7	.	0.0203	0.0107	65.32

L. Thomsons	Vegetated	1	.	.	.	.	0.0255	0.0000	100.00
L. Thomsons	Vegetated	2	20.3	5.3	2.1	18.3	0.0253	0.0130	66.01
L. Thomsons	Vegetated	2	1.7	0.7	0.9	.	0.0142	0.0095	60.00
L. Thomsons	Vegetated	2	.	.	.	.	.	.	.
L. Thomsons	Vegetated	3	1.0	0.0	1.0	.	.	.	.
L. Thomsons	Vegetated	3	.	.	.	.	.	.	.
L. Thomsons	Vegetated	3	.	.	.	.	.	.	.
L. Thomsons	Vegetated	4	.	.	.	.	.	.	.
L. Thomsons	Vegetated	4	.	.	.	.	.	.	.
L. Thomsons	Vegetated	4	.	.	.	.	.	.	.
L. Mt. Brown	Open	1	4.3	1.3	0.9	0.9	0.0132	0.0020	86.89
L. Mt. Brown	Open	1	.	.	.	.	.	.	.
L. Mt. Brown	Open	1	.	.	.	.	.	.	.
L. Mt. Brown	Open	2	5.3	2.0	1.1	65.0	0.0258	0.0232	52.55
L. Mt. Brown	Open	2	8.3	2.0	0.6	.	0.0357	0.0318	52.96
L. Mt. Brown	Open	2	9.0	0.3	0.4	.	0.0380	0.0175	68.47
L. Mt. Brown	Open	3	6.0	0.7	1.7	10.3	0.0205	0.0295	41.00
L. Mt. Brown	Open	3	14.3	6.0	1.7	.	0.0300	0.0355	45.80
L. Mt. Brown	Open	3	4.3	2.7	1.7	.	0.0403	0.0415	49.24
L. Mt. Brown	Open	4	2.0	16.7	3.1	76.9	0.1020	0.1658	38.10
L. Mt. Brown	Open	4	2.7	0.7	1.8	.	0.0255	0.0445	36.43
L. Mt. Brown	Open	4	11.3	2.0	1.8	.	0.0370	0.0258	58.96
L. Mt. Brown	Vegetated	1	20.0	1.3	3.5	181.8	0.0223	0.0032	87.25
L. Mt. Brown	Vegetated	1	31.3	1.3	1.1	.	0.0290	0.0215	57.43
L. Mt. Brown	Vegetated	1	.	.	.	.	0.0440	0.0205	68.22
L. Mt. Brown	Vegetated	2	22.0	1.7	0.3	8.9	0.2065	0.1785	53.64
L. Mt. Brown	Vegetated	2	8.0	2.0	1.6	.	0.1513	0.0895	62.82
L. Mt. Brown	Vegetated	2	7.3	1.0	1.0	.	0.1135	0.0785	59.11
L. Mt. Brown	Vegetated	3	17.3	3.3	2.6	39.8	0.0395	0.0440	47.31
L. Mt. Brown	Vegetated	3	2.3	7.3	4.2	.	0.1375	0.1270	51.98
L. Mt. Brown	Vegetated	3	31.3	2.0	2.3	.	.	.	.
L. Mt. Brown	Vegetated	4	0.0	1.0	0.4	26.7	0.1923	0.1667	53.55
L. Mt. Brown	Vegetated	4	2.7	14.3	3.3	.	0.2290	0.2400	48.83
L. Mt. Brown	Vegetated	4	9.7	9.7	2.1	.	0.0797	0.0485	62.18
L. Balannup	Open	1	.	.	.	.	.	.	.
L. Balannup	Open	1	.	.	.	.	.	.	.
L. Balannup	Open	1	.	.	.	.	.	.	.
L. Balannup	Open	2	1.3	1.0	0.6	8.7	0.0300	0.0045	86.96
L. Balannup	Open	2	2.0	3.3	1.8	.	0.0097	0.0020	82.98
L. Balannup	Open	2	0.7	5.3	2.2	.	0.0207	0.0185	52.87
L. Balannup	Open	3	1.0	1.7	1.8	1.2	0.0218	0.0130	62.59
L. Balannup	Open	3	0.0	0.0	0.5	.	0.0147	0.0178	45.38
L. Balannup	Open	3	0.7	4.3	1.7	.	.	.	.
L. Balannup	Open	4	.	.	.	.	.	.	.
L. Balannup	Open	4	.	.	.	.	.	.	.
L. Balannup	Open	4	.	.	.	.	.	.	.
L. Balannup	Vegetated	1	.	.	.	.	.	.	.
L. Balannup	Vegetated	1	.	.	.	.	.	.	.
L. Balannup	Vegetated	1	.	.	.	.	.	.	.

L. Balannup	Vegetated	2	3.3	2.3	3.2	19.9	0.0035	0.0000	100.00
L. Balannup	Vegetated	2	0.0	6.0	0.9	.	0.0060	0.0007	88.89
L. Balannup	Vegetated	2	0.0	8.0	1.1	.	0.0157	0.0058	73.26
L. Balannup	Vegetated	3	2.7	3.3	2.7	11.7	0.0180	0.0145	55.38
L. Balannup	Vegetated	3	.	.	.	.	.	.	.
L. Balannup	Vegetated	3	.	.	.	.	.	.	.
L. Balannup	Vegetated	4	.	.	.	.	.	.	.
L. Balannup	Vegetated	4	.	.	.	.	.	.	.
L. Balannup	Vegetated	4	.	.	.	.	.	.	.

APPENDIX B

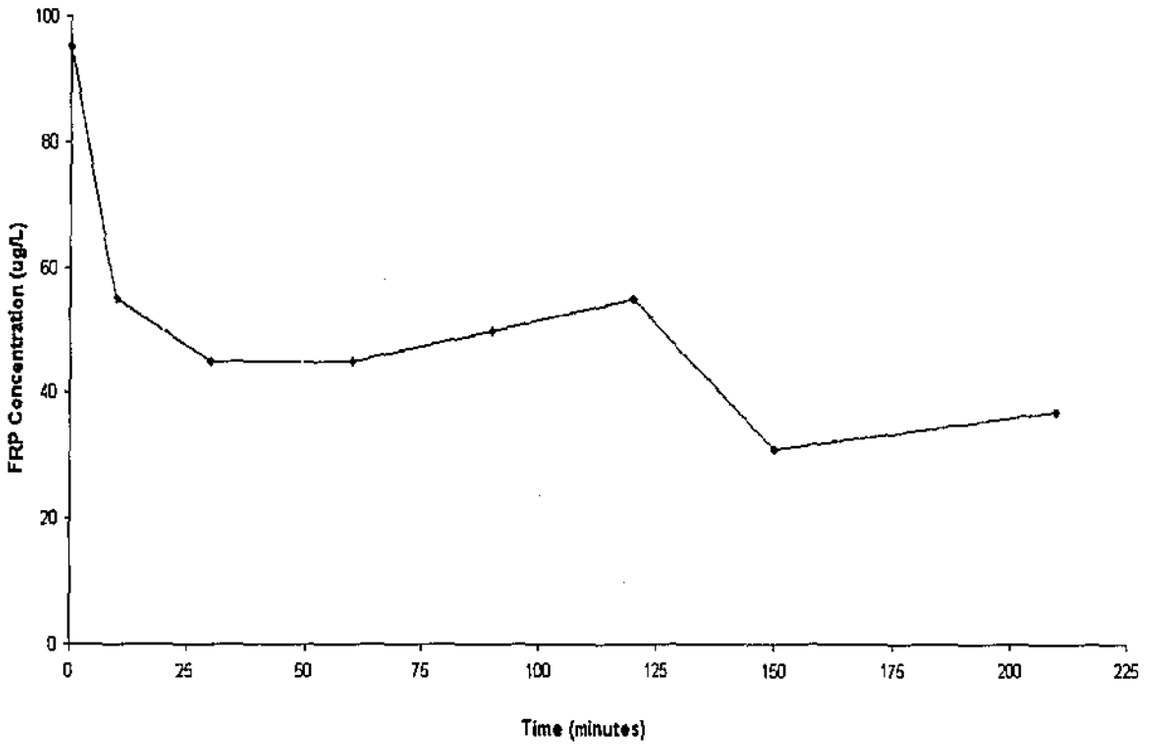
Biofilm FRP Uptake Kinetic Experiments: Pilot Study

APPENDIX B

Pilot Study for Biofilm FRP Uptake Kinetic Experiments

The pilot study was conducted with one uptake tank at $100ugL^{-1}$ and PAR intensity provided at $10.3umolm^{-2}sec^{-1}$. Plates had been deployed in Hammond Road CWS for 6 weeks.

The pilot study was used to determine sample extraction times and the total experiment time. Th greatest uptake occurred within the first 30 minutes. The Pre-test indicated that a 2-hour period with samples taken at 0, 5, 10, 20, 35, 55, 85 and 120 minutes would be appropriate for the biofilm FRP uptake kinetic experiments.



APPENDIX C

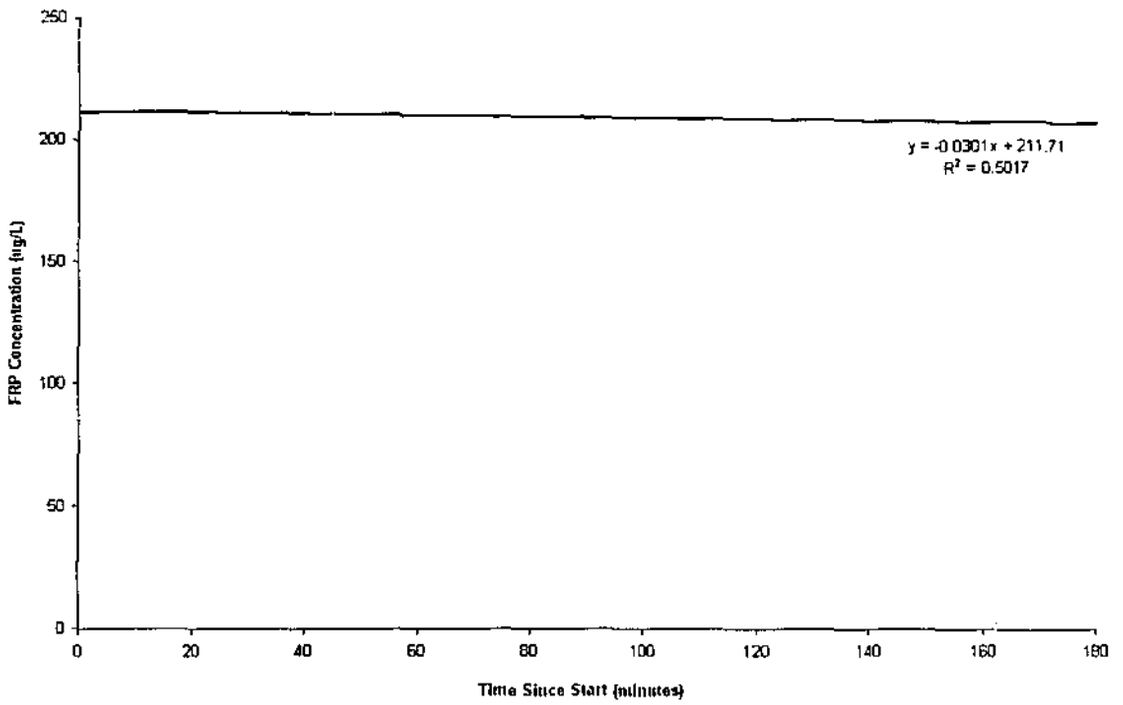
Biofilm FRP Uptake Kinetic Experiments: High PAR Intensity

APPENDIX C

High PAR Uptake Kinetic Test Results

FRP uptake was determined at 200ugL^{-1} with 5 replicate samples with PAR intensity provided at 10.3umolm^{-2}sec^{-1}. An additional sample extraction was taken at 180minutes, with all other sample extraction timings remaining the same as the main FRP uptake experiment. The plates used had been in Hammond Road CWS for a period of 4 weeks. Analysis of the biomass from each tank was then conducted.

The uptake rate appeared similar to experienced in the main FRP uptake experiment. When the uptake was normalised to weight, the uptake rate was confirmed as being similar. This indicated that the FRP uptake rate of the biofilm collected in the main FRP uptake experiments was not limited by light.



FRP Concentration	Mean Tank Uptake Rate (μghr^{-1})	Mean Organic Biomass (mg)	Uptake Rate per mg Organic Biomass per hour. ($\mu gmg^{-1}hr^{-1}$)
200μgL^{-1}	3.612	13.7	**0.263**

Mean Tank Uptake Rate, Mean Organic Biomass and Mean Uptake Rate to Biomass for the High Light Orthophosphate Uptake Kinetic Experiment.

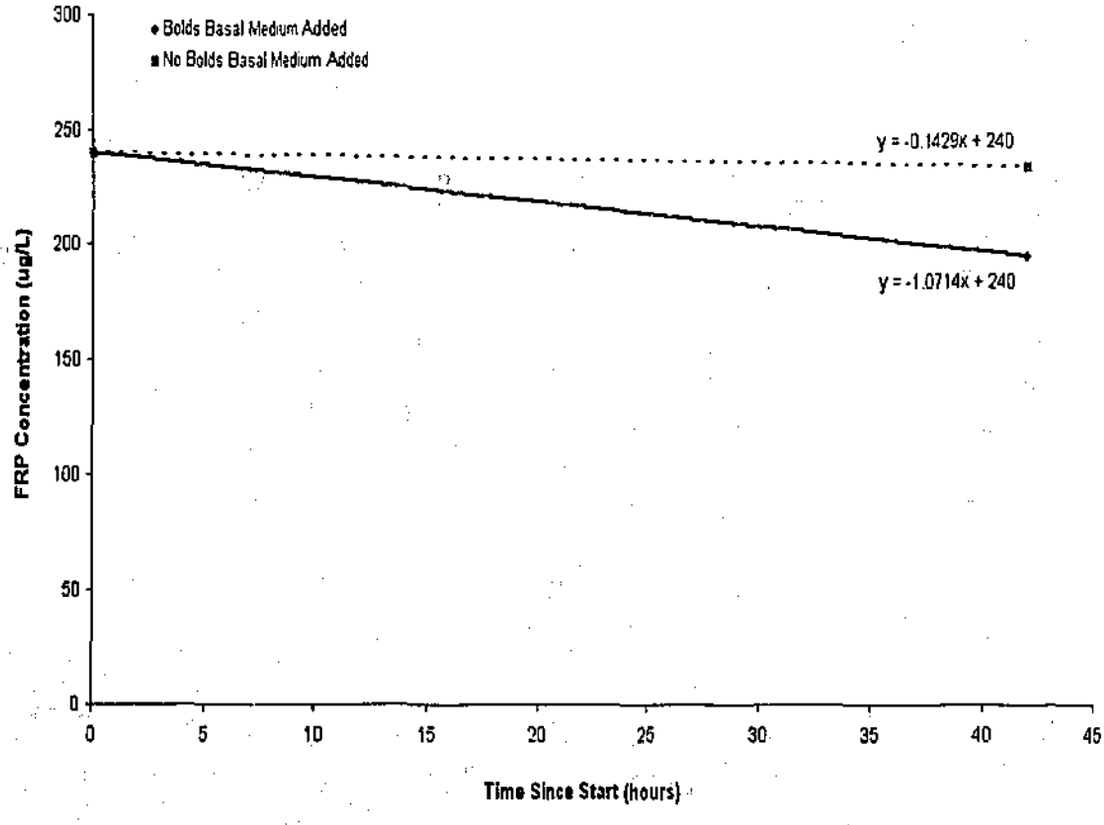
APPENDIX D

Biofilm FRP Uptake Kinetic Experiments: BBM

APPENDIX D

BBM Uptake Kinetic Experiments

The effects of BBM on FRP uptake was tested with limited time and a lack of biofilm plates in Hammond Road CWS. As a result of this, no replication was possible for the experiment. The experiment was conducted using two FRP uptake tanks at 250ugL^{-1}, one containing BBM and the other minus BBM, over a 42-hour period with the PAR intensity provided at 10.3umolm^{-2}sec^{-1} . Samples were extracted at 0hrs and 42hrs. The system containing BBM had a loss of FRP greater than the system in which BBM was absent. It was therefore assumed that the lower rate of FRP removal in the system without BBM was due to the biofilm being nutrient limited. The experiment indicated that there was no indication that the BBM interfered with the FRP uptake by biofilm during the other FRP uptake experiments.



APPENDIX E

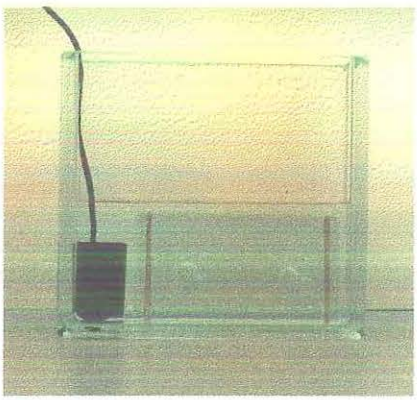
Dye Circulation Test

APPENDIX E

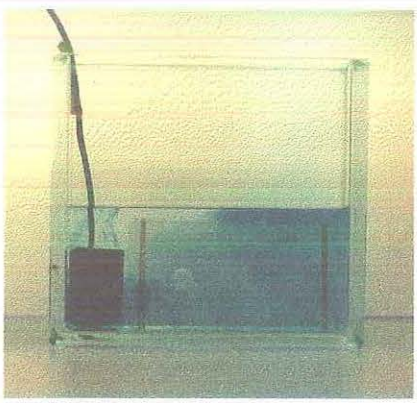
Dye Circulation Test.

The uptake cell prior to the addition of the blue dye is shown by (A). Blue dye was pipetted from behind the water pump from above (B). As shown, the dye is circulated from the pump along the base of the cell, before being circulated between the plate pairs and along the top of the water until the dye has completely circulated (C). The sequence covers a time of within 0 and 5 seconds.

A)



B)



C)